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14. ABSTRACT: The structural re-modeling of the mammalian nucleus is a key feature of cancer cells. Such reorganization of the nucleus impacts the genomic integrity of the cell. Our focus is on the genetic alterations that affect the telomeres (the ends of chromosomes) in breast cancer. In this study, we wished to determine if mutations in either of the two main breast cancer susceptibility genes, known as BRCA1 and BRCA2 can influence the way in which telomeres are organized. To do this, we studied the three-dimensional (3D) organization of telomeres in breast cancers derived from BRCA1 and BRCA2 gene mutation carriers. We used breast cancers arising in non-BRCA1/2 mutation carriers as controls. In addition, we studied three cancer cell lines derived from BRCA1-, BRCA2- and non-carriers to see if we found the same effect. There were two main measures- the length of the telomeres and the aggregation of the telomeres (i.e. to what extent telomeres were found 'stuck together')- this is usually the result of chromosomes with broken ends becoming fused. To summarize, the results were not conclusive. It was clear that the BRCA1 and BRCA2 cell lines had shorter telomeres and more aggregations compared with the controls, but in the tumors, the results were less clear. There was, however, a non-significant trend in the same direction as observed in the cell lines. This work is now continuing, and we aim to submit this work for publication in the coming year. The role of MYC in this process is of particular interest, as many BRCA1 tumours show amplification of MYC, and MYC is known to interact with other proteins in the maintenance of telomere length. We are currently analyzing the same set of tumours for MYC amplification by FISH.

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INTRODUCTION:

Telomeres are the ends of chromosomes. By capping the chromosomes, they are responsible for chromosomal integrity to prevent genomic instability. Two-dimensional studies of interphase nuclei have been unable to precisely describe the position of telomeres in either normal or cancer cells. Recent three-dimensional (3-D) imaging of the mammalian interphase nucleus, followed by highly sophisticated computerized image analysis, has resulted in the description of organized, dynamic telomere territories (TT) in normal cells. These TT are cell-cycle dependent: in G2, the telomeres are organized into telomeric disc (TD). TT, and in particular, the TD, are disrupted as a result of c-Myc deregulation[1]. In some tumor cells, the same disruption of TT is seen. Notably, *BRCA1*-related breast cancers often show c-Myc deregulation[2]: the role of c-Myc alterations in *BRCA2*-related breast cancer is less clear, but in general, more aggressive breast cancers are more likely than quiescent tumors to have elevated levels of c-Myc protein. Thus, we postulate that disorganization of telomeres in *BRCA1* and/or *BRCA2*-related breast cancer is a key step in the development of these cancers.

BODY:

Task 1: To conduct telomere hybridizations and examination of 3D telomeric organization
This work was completed.

Task 2: To determine whether regulatable BRCA1 and BRCA2 mutations alter the levels of telomeric aggregation and genomic instability in 3D nuclei
This work was commenced, is on-going but has not been completed.

Task 3: To examine whether BRCA1 and BRCA2 mutations in conjunction with c-Myc deregulation accelerate aggregation and genomic instability.
This work is just starting now, and will be completed over the next year. Grants have been awarded on the basis of our preliminary findings that will allow us to complete this work.

KEY RESEARCH ACCOMPLISHMENTS:

- More than 25 breast tumours were analysed in detail
- Cell lines with and without BRCA1 and BRCA2 mutations were also analysed.
- BRCA1 and BRCA2 mutations appear to influence telomere structure
- Further studies on this subject have been made possible because of this award.

REPORTABLE OUTCOMES:

1. Presentations (by Soumya Panigrahi):

September 9-13, 2006
Sherif F. Louis, Bart Vermolen, Soumya Panigrahi, William D. Foulkes, Yuval Garini and Sabine Mai

Title: *Telomeric aggregates in c-Myc-dependent genomic instability and tumorigenesis in general.*
Fifth Annual Symposium of Hormonal Carcinogenesis
Montpellier, FRANCE

May 11-14, 2006
Panigrahi S, Wark L, Vermolen B, Watson P, Snell L, Chuang A, Kotar K, Garini Y, Foulkes W, Mai S.
Title: *Three Dimensional Organization of Telomeres in Human Breast Cancer.*
Canadian Telomere Symposium
Calgary, CANADA

October 20-21, 2005
CME: *The First International Symposium on the Hereditary Breast and Ovarian Cancer Susceptibility Genes*
Montreal, CANADA

June 2004
Panigrahi et al.
Title: *Three Dimensional Organizations of Telomeres in Human Breast Cancer*
(Poster presentation)
Canadian Telomere Group

2. Cell lines: we developed 2 new BRCA1 cell lines to allow us to look at telomere organization in BRCA1 heterozygotes.

3. Sabine Mai (co-PI) was awarded a Susan G Komen breast cancer grant (with the PI) on the basis of the preliminary work done here.

4. Members of the research community have visited Dr. Mai's lab on CIHR-funded short training grants; some of these awards were made possible because of this and similar work that has been carried out in Dr. Mai's laboratory.

5. Patent: Mai, S., Chuang, T., Moshir, S, Garini, Y. Method of monitoring genomic instability by 3D microscopy and analysis. – Serial No. 9157-51.

CONCLUSION:

This study has shown that cell lines derived from BRCA1 and BRCA2 carriers are statistically significantly more likely to show telomere aggregations than cell lines derived from non-carriers. Results form formalin-fixed, paraffin-embedded breast tumors support these findings, but the results are not conclusive. Our main focus now will be to study more tumors, and to look at the interaction between MYC and BRCA1 or BRCA2 in determining the extent of telomere aggregations. This work is important, as recent research suggests that MYC and BRCA1 may have an important joint role in sporadic breast cancer.[3]

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APPENDICES:

Journal Articles

Garini Yand Mai S. The Significance of Telomeric Aggregates in the Interphase Nuclei of tumor Cells. Journal of Cellular biochemistry, 97: 904-915, 2006.

Caporali A, Wark L, Vermolen BJ, Garini Y, Mai S. Telomeric aggregates and end-to-end chromosomal fusions require myc box II. Oncogene, 1-6, 2006.

Mai S and Garini Y. Oncogenic remodeling of the Three-dimensional organization of the interphase nucleus. Cell cycle, 4:10, 1327-1331, 2005.

Vermolen BJ, Garini Y, Mai S, Mougey V, Fest T, Chuang TC-Y, Chuang AY-C, Wark L, Young IT. Characterizing the three-dimensional organisation of telomeres. Int. Society for Analytical Cytology, 67A:144-150, 2005.

Louis SF, Vermolen BJ, Garini Y, Young IT, Guffei A, Lichtensztejn Z, Kuttler F, Chuang TC-Y, Moshir S, Mougey V, Chuang AY-C, Kerr PD, Fest T, Boukamp P, Mai S. c-Myc induces chromosomal rearrangements through telomere and chromosome remodeling in the interphase nucleus. Proc. of the National Academy of Science, 102; 27: 9613-9618, 2005.

Chuang TC-Y, Moshir S, Garini Y, Chuang AY-C, Young IT, Vermolen B, van den Doel R, Mougey V, Perrin M, Braun M, Kerr PD, Fest T, Boukamp P, Mai S. The three-dimensional organisation of telomeres in the nucleus of mammalian cells. BMC Biology, 1-8, 2004.

Journal of Cellular Biochemistry 97:904–915 (2006)

The Significance of Telomeric Aggregates in the Interphase Nuclei of Tumor Cells

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Abstract Telomeres are TTAGGG repetitive motifs found at the ends of vertebrate chromosomes. In humans, telomeres are protected by shelterin, a complex of six proteins (de Lange [2005] Genes Dev. 19: 2100–2110). Since (Müller [1938] Collecting Net. 13: 181–198; McClintock [1941] Genetics 26: 234–282), their function in maintaining chromosome stability has been intensively studied. This interest, especially in cancer biology, stems from the fact that telomere dysfunction is linked to genomic instability and tumorigenesis (Gisselsson et al. [2001] Proc. Natl. Acad. Sci. USA 98: 12683–12688; Deng et al. [2003] Genes Chromosomes Cancer 37: 92–97; DePinho and Polyak [2004] Nat. Genetics 36: 932–934; Meeker et al. [2004] Clin. Cancer Res. 10: 3317–3326). In the present overview, we will discuss the role of telomeres in genome stability, recent findings on three-dimensional (3D) changes of telomeres in tumor interphase nuclei, and outline future avenues of research. J. Cell. Biochem. 97: 904–915, 2006.

Key words: oncogenes; 3D nucleus; genomic instability; telomeres; telomeric aggregates; chromosomes; breakage-bridge-fusion cycle; genomic instability

SIGNIFICANT EARLY WORK ON TELOMERE BIOLOGY

Müller [1938] and McClintock [1941] were the first to observe breakage-bridge-fusion (BBF) cycles. These are cycles where chromosomal end-to-end fusions contribute to the onset of chromosomal rearrangements and genomic instability. Studying broken chromosomes in *Zea mays*, McClintock [1942] observed the formation of dicentric and ring chromosomes, rearrangements, terminal deletions, and chromatin bridges at anaphase that then broke apart unequally ("non-median breaks"). She also observed continuous cycles of these events, that is cycles of new fusions followed by new breakages in the following anaphases [McClintock, 1941, 1942]. Focusing on chromosome 9 in *Zea mays*, she was able to follow distinctive types of variegation and to link them to specific rearrangements on previously broken chromosomes. Broken chromosomes were then able to fuse with sister chromatids or with other chromosomes. This affected not only kernel color but also starch formation, growth conditions, and propagation of the plants [McClintock, 1942].

The questions McClintock asked then are still valid today. "(1) Must two chromosomes or more chromosomes be in intimate contact at the time of breakage in order that fusions may occur? (2) If no intimate contact is necessary at the time of breakage, are the broken ends "unsaturated," that is capable of fusion with any other

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unsaturated broken end? (3) If question (2) can be answered in the affirmative, what forces are involved which lead to the contact and subsequent fusion of the two unsaturated ends? Likewise, (4) how long will these broken ends remain unsaturated, that is, capable of fusion?" [McClintock, 1942]. We found it important to use Barbara McClintock's own words to summarize some of the key questions in the field. Please note that these questions were formulated in 1942. Today, the concept of chromosomal localization is still under intense debate with respect to specific rearrangement of chromosomes. The "unsaturated ends" are indeed broken chromosomal ends that are free of telomeres and therefore able to fuse with sister chromatids or other chromosomes, and yes, chromosome ends can "be healed."

STRUCTURAL ORGANIZATION OF TELOMERES IN MAMMALIAN NUCLEI

Most studies with telomeres have been performed on metaphase chromosomes. Metaphase chromosomes reflect events that occurred prior to the metaphase being examined and, with respect to some aberrations, researchers infer from studying the metaphase chromosomes that 'telomere dysfunctions' were likely. For example, unbalanced translocations, dicentric chromosomes, and terminally deleted chromosomes suggest a defect in telomeres that may involve capping defects, DNA damage affecting the telomeric ends, oncogene activation or other stimuli [Artandi et al., 2000; Gisselsson et al., 2001; Lo et al., 2002; Deng et al., 2003; Murnane and Sabatier, 2004; Louis et al., 2005].

Advances in imaging allow us to now focus on the events that occur prior to the metaphase, namely in preceding cell cycle stages of interphase nuclei. While two-dimensional (2D) imaging of nuclei did not allow us to visualize the spatial organization of telomeres, three-dimensional (3D) and live cell imaging permit the analysis of the structural organization of telomeres in the nucleus of mammalian cells. Studies in recent years have then shown us that telomeres in normal nuclei have a dynamic cell cycle- and tissue-dependent organization. For example, in G0/G1 nuclei, telomeres are widely distributed throughout the whole nuclear space [Weierich et al., 2003; Chuang et al., 2004]. Measurements of telomere positions in the 3D space of primary mouse lymphocyte nuclei have given a precise value to telomeres in this phase of the cell cycle. The a/c ratio indicates that telomeric positions in interphase nuclei is small in G0/G1 lymphocytes, and one usually measures values of 1.4 ± 0.1 [Vermolen et al., 2005a]. This number is indicative of the distribution of telomeres throughout the entire nuclear space of primary lymphocytes, which is roughly spherical. Similarly, in S phase, the a/c ratio is small (1.5 ± 0.2; [Vermolen et al., 2005a]). The nuclear distribution of telomeres changes when cells enter into G2: telomeres align in the center of the nucleus and form a telomeric disk [Chuang et al., 2004]. At this time, the a/c ratio is large due to the organization of the telomeres in a disk-like volume, and the a/c ratio measurements usually are 14 ± 2 [Vermolen et al., 2005a]. Telomere dynamics in interphase nuclei of human osteosarcoma (U2OS), human cervical carcinoma (HeLa), and mouse MS5 cells has been carefully measured by live cell imaging approaches. Long ranging as well as short movements were observed over a time period of 20 min [Molenaar et al., 2003]. Telomere dynamics has also been observed in interphase nuclei of human keratinocytes [Ermler et al., 2004]. Telomere movement is not only dependent on cell cycle but also on cell shape [Chuang et al., 2004; Ermler et al., 2004]. Thus, we conclude that telomeres are not static in mammalian nuclei but perform cell cycle and cell-type specific movements.

Another important feature of telomeres in normal interphase nuclei is the fact that the telomeres do not overlap. Each telomere of a normal nucleus is found in its specific 3D space and does not form clusters or aggregates with other telomeres [Chuang et al., 2004]. Normal cells have a limited life span [Hayflick, 1965]. Their mitotic clock is linked to telomere length. Telomere length is known to be shortening linearly with each cell division (approximately 50–200 base pairs per division [Lansdorp, 2000]). When the telomeres become too short, normal cells will eventually stop division cycles and enter into a state of replicative arrest that is also called senescence. The senescent phenotype has been extensively studied [for review, see Campisi, 2000]. Senescence is bypassed during tumor development [Campisi, 2000; Romanov et al., 2001].

TELOMERE ORGANIZATION IN TUMOR CELLS

Telomeres in tumor cells are different from telomeres in normal cells; they are generally shorter, even critically short [Vukovic et al., 2003; Meeker et al., 2004]. However, they may also be elongated or different subpopulations of telomere lengths may be present [Meeker et al., 2004]. It was shown that telomeres in tumor cells commonly manifest telomere dysfunction, and chromosomal aberrations indicative of these defects are observed. Telomerase is activated in 85% of the tumors, while it is not present in the rest of the tumors, some of which have demonstrated alternative lengthening of telomeres (ALT) [Muntoni and Reddel, 2005].

A remarkable difference between normal and tumor cells becomes apparent when 3D imaging approaches are applied. 3D imaging revealed a specific 3D telomeric signature for tumor cells. In contrast to the non-overlapping nature of telomeres in normal nuclei, telomeres of tumor nuclei tend to form aggregates. Various numbers and sizes of such telomeric aggregates (TAs) can be found in tumor nuclei [Chuang et al., 2004]. The formation of TAs is independent of telomere length and telomerase activity [Louis et al., 2005].

There are at least two types of telomeric dysfunction in tumor cells. One type of telomere dysfunction involves critically short telomeres [DePinho and Polyak, 2004]. The other one involves the formation of TAs and is independent of telomere size or telomerase activity [Chuang et al., 2004; Louis et al., 2005]. Both types of telomeric dysfunction can lead to BBF cycles that contribute to deletions, gene amplification, non-reciprocal translocation, and overall genetic changes that are associated with tumorigenesis [Artandi et al., 2000; DePinho and Polyak, 2004; Murnane and Sabatier, 2004].

MEASUREMENT OF TELOMERE DYSFUNCTION (3D VOLUMES AND POSITIONS)

Quantitative measurement of the telomeres parameters is based on 3D data that are usually captured by acquiring many optical sections of the nucleus with a high numerical aperture oil-immersed objective lens followed by an appropriate deconvolution algorithm. The most reliable one uses constrained iterative deconvolution [Schaefer et al., 2001; Vermolen et al., 2005b]. Telomere measurements are done with a special algorithm and software package that we developed, TeloView^{TM} [Chuang et al., 2004; Vermolen et al., 2005a]. First, the position of each telomere is identified by using a threshold. Then, the center of gravity and the integrated intensity of each telomere are calculated. The integrated intensity of each telomere is the appropriate parameter for determining the length of the telomere, or the telomere copy number, which estimates the number of telomeres that are taking part in an aggregate. Aggregates are easily observed when looking at a 3D visualization of the nucleus and it can be quantitatively calculated by analyzing the integrated intensity of each telomere (Fig. 1).

IMPACT OF TELOMERE AGGREGATES ON CHROMOSOMAL ORGANIZATION

It is not just a transient aberration in the 3D organization of the nucleus when telomeres aggregate. Since some of the aggregates represent fusions, dicentric chromosomes can form. These end-to-end fused chromosomes cannot appropriately separate during cell division, but will first generate anaphase bridges and then break apart, leaving one chromosome too short (with a terminal deletion) and the other one with

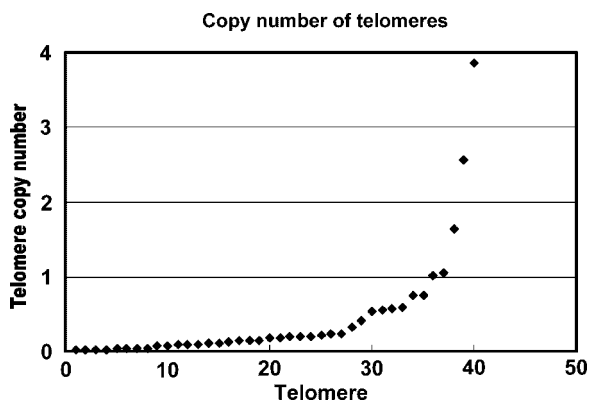

Fig. 1. Histogram illustrating the concept of telomeric aggregates (TAs) and their quantitative analysis. This histogram demonstrates how TAs are found using TeloView^{TM} [Vermolen et al., 2005a]. Each point represents the copy number of a telomere that is found in the nucleus. The intensity of an average telomere is calculated by analyzing the smaller telomeres in the nucleus (which are the majority of telomeres). See the change in the graph slope at about telomere number 37. All the telomeres smaller than telomere number 37 are interpreted as single copies while telomeres that are larger are interpreted as aggregated copies. The copy number is calculated by dividing the integrated intensity of each telomere by that of telomere number 37. The telomeres are sorted for convenience from smallest to largest (based on their integrated intensity).

a new piece (generating an unbalanced translocation). Both chromosomes are 'new' structures and both are unable to persist the way they were left after this cell division. Since both new chromosomes have telomere free ends and represent a double-strand break, they will each find a new chromosome partner, and they will fuse with it to heal their broken ends. This series of events is termed a BBF cycle and goes back to Müller and McClintock's seminal findings [Müller, 1938; McClintock, 1941]. Importantly, a BBF cycle is not a single event. One BBF cycle initiates the next and so forth until no more free ends persist to permit fusions with other chromosomes [McClintock, 1941, 1942; Louis et al., 2005].

Once aggregates form and fusions occur, BBF cycles result and with such BBF cycles, the genetic information of the chromosomes will be remodeled [Louis et al., 2005]. TAs and fusions are different from the reversible telomeric associations that have been reported for Chinese hamster embryonic cells [Slijepcevic et al., 2000]. Which events lead to such telomere-mediated nuclear remodeling? We have studied oncogenic remodeling of the 3D telomere organization. The deregulation of the oncoprotein c-Myc was able to remodel the telomeric organization from non-overlapping telomeres to TAs of various numbers and sizes [Louis et al., 2005]. A single deregulation event of c-Myc, where the oncogene was overexpressed in the nucleus for 2 h, was sufficient to initiate the formation of TAs. Moreover, TAs/fusions caused the formation of dicentric, end-to-end fused chromosomes. The latter generated anaphase bridges and broke apart as anaphase progressed, leaving behind terminal deletions and unbalanced translocations. Two hours of c-Myc deregulation initiated three BBF cycles. Twelve hours of c-Myc deregulation led to five such cycles. Thus, the time of c-Myc deregulation was directly proportional to the number of BBF cycles observed [Louis et al., 2005]. The scoring of chromosomal aberrations over a 120-h period documented the BBF cycles: from fusion to breakage with terminal deletions and non-reciprocal translocations to telomere-free ends and new fusions (ibid).

WHICH ABERRATIONS ARE GENERATED WHEN TELOMERES ARE REMODELED IN THE 3D SPACE OF THE NUCLEUS?

Two sets of parallel experiments involving chromosome painting to determine the 3D organization of chromosomes in interphase nuclei and spectral karyotyping (SKY) of metaphase chromosomes were carried out to examine the effects of TA formation on chromosomal positions and aberrations [Louis et al., 2005]. SKY data showed non-random chromosomal rearrangements affecting chromosomes $5+13$, $7+10$, $7+17$. Other chromosomes were sometimes, but not regularly involved and judged as random aberrations. When examining the positions of chromosomes $5+13$, $7+10$, and $7+17$ in interphase nuclei, we found no overlap between these pairs prior to Myc activation, while they changed their positions over the time course of c-Myc deregulation and showed substantial overlap [Louis et al., 2005].

MEASUREMENTS OF CHROMOSOMAL OVERLAPS IN THE INTERPHASE NUCLEUS

Chromosomal overlaps measurements are performed after 3D image acquisition and constrained iterative deconvolution. First, the 3D boundary of the nucleus is determined based on the DAPI counterstain image. Within this volume, a threshold level is determined for each chromosome and the total volume V_1 and V_2 of each chromosome pair is calculated (by counting only the voxels that has an intensity value above the threshold). The total volume that is occupied by both chromosome pairs is also measured (V_0). By dividing V_0 by the total volume of each one of the chromosome pairs, the relative overlap ratio is calculated, V_0/V_1 and V_0/V_2. By following the same procedure for each time point since c-Myc deregulation, we finally get the relative overlap as a function of time.

SIGNIFICANCE OF OVERLAPPING CHROMOSOMES

Chromosomal overlap is a problem for genome stability if the overlapping chromosomes fuse at their telomeric ends or are involved in illegitimate recombination events. TAs brings chromosomes into close vicinity. If TAs represent fusions, then BBF cycles will occur. This was found after experimentally-induced c-Myc deregulation [Louis et al., 2005; Mai and Garini, 2005].

There are two possibilities for the initiation of BBF cycles after TA formation and chromosome overlap. The occurrence of non-random chromosomal aberrations suggests either a non-random formation of chromosomal overlaps

resulting in end-to-end chromosomal fusions. Alternatively, one may argue that there is a non-random occurrence of TA formation resulting in chromosomal overlaps and causing the initiation of BBF cycles. At the present time, we cannot distinguish between both possibilities and both remodeling events may coexist.

TAs AND TUMORS

Genomic instability is viewed as an event through which genetic changes occur or have occurred [Hanahan and Weinberg, 2000; Gollin, 2005; Mitelman et al., 2005]. These changes can be structural and numerical, and this is the classical view of genomic instability. We would like to expand this view and include epigenetic changes that coincide with genetic alterations and/or precede them, point mutations, and alterations in nuclear organization that affect the genome. Organizational changes in the 3D space of the nucleus need to be considered as an important factor not only in tumors but also much earlier that is during the initiation of genomic instability and the establishment of tumorigenic potential.

The analysis of primary tumors revealed that TAs are common [Chuang et al., 2004]. Various cell types and tissues were examined, including primary head and neck cancer, primary mouse plasmacytoma, human neuroblastoma, and colon carcinoma cell lines [Chuang et al., 2004]. While normal cells do not show TAs, tumor cells (primary tumor cells and tumor cell lines) consistently display TAs (Fig. 2).

Importantly, being a feature of tumor cells makes one wonder if such changes in the telomeric organization of the interphase nucleus do not occur earlier, that is when cells become tumorigenic. Early data suggest that this is indeed the case. For example, in cervical cancer, non-invasive lesions, such as CIN I, show TAs in some of cells (Fig. 3). During the development of mouse plasmacytoma, early plasmacytotic foci display TAs in a subpopulation of the foci (Fig. 4). Additional analyses are ongoing and will help us understand the earliest

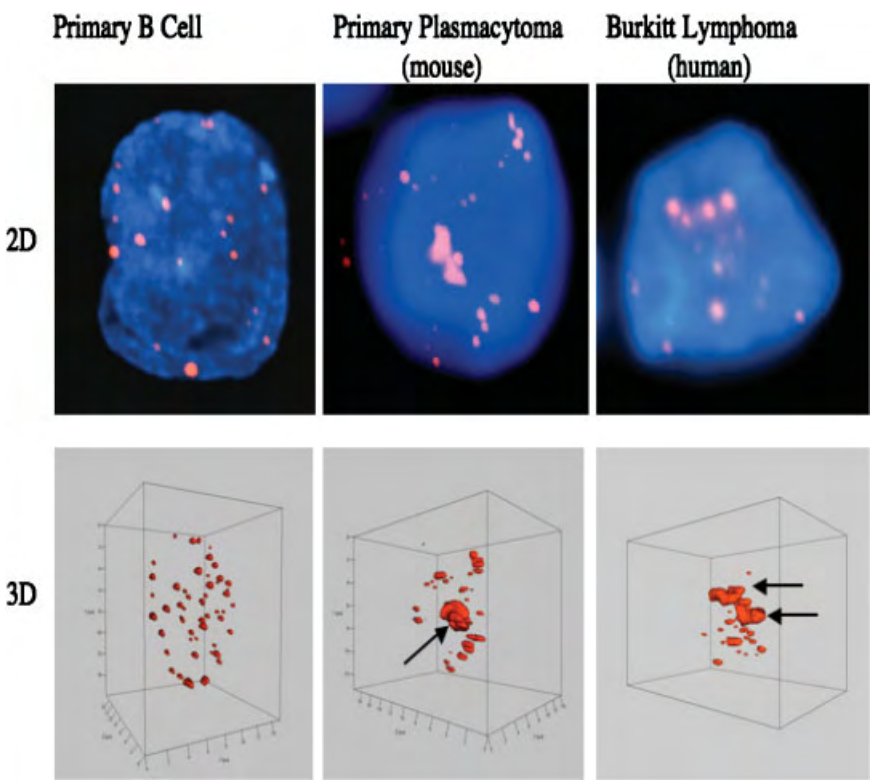

Fig. 2. Telomere organization in primary nuclei of a B cell, a primary mouse plasmacytoma and a Burkitt lymphoma line (Raji). The **top panel** shows two-dimensional (2D) representations of the above nuclei; the **bottom panel** shows the three-dimensional (3D) organization of telomeres in the above nuclei. Telomeres are shown in red, nuclei are shown in blue. Arrows point to TAs. Hybridizations were preformed as described [Chuang et al., 2004; Louis et al., 2005].

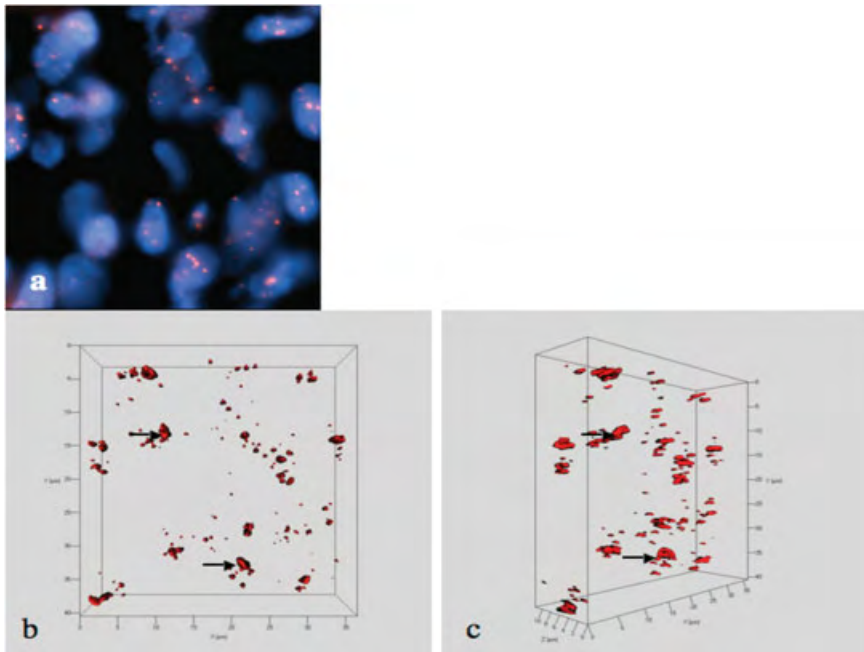

Fig. 3. Telomere organization in cervical biopsy tissue of a CIN 1 lesion. **a**: 2D image of a section showing the identical nuclei (blue) and their telomeric signals (red) that are shown in (**b**) and (**c**) as 3D images. Black arrows point to TAs that are observed in some of the cells. Frozen sections of 5-μm thickness were hybridized as described [Chuang et al., 2004; Louis et al., 2005].

time point during tumor development in vivo that show TA formation. In vitro, in a model of c-Myc-induced genomic instability in PreB and Ba/F3 mouse lymphocytes, we have shown that c-Myc deregulation elicits TA formation within 12 h [Louis et al., 2005]. Additional studies propose even earlier time points (unpublished data). Taken together, the above data indicate that the formation of TAs is an intrinsic factor in the transformation of the normal cell into a malignant one. Therefore, in the future, the knowledge of TA formation during tumor development can be used as a diagnostic tool and for monitoring of treatment success.

MECHANISMS OF TA FORMATION

How do these aggregates form? This is an area that requires intense research. At this point, nothing is known about the mechanisms that cause TA formation. One may speculate that one of the shelterin proteins [de Lange, 2005] is causally involved in TA formation. However, this has not been demonstrated in tumor models. We know from studies of de Lange and colleagues that the absence of TRF2 leads to the formation of telomeric fusions which lead to cell death and senescence [van Steensel et al., 1998; Celli and de Lange, 2005]. Whether these fusions involve TA formation and can be linked to genomic instability and cancer has not been investigated.

BOVERI'S LEGACY: IN SEARCH OF THE MECHANISMS THAT REGULATE ABERRANT NUCLEAR AND GENOMIC ORGANIZATION

Although we described the formation of TAs in tumors and after c-Myc deregulation for the first time [Chuang et al., 2004; Louis et al., 2005], the concept of the nucleus and its chromosomal order has been studied long before. Theodore Boveri (1862–1915) was the first researcher who linked nuclear organization and genome stability. Studying *Ascaris* and sea urchin eggs, he described for the first time 'chromosomal regions' ('chromosome territories' [Cremer and Cremer, 2001]). Chromosomal regions are regions within the 3D nuclear space in which chromosomes tend to be found in normal cells. Boveri also noted that an aberrant chromosome constitution leads to aberrant cell division cycles and mis-segregation of chromosomes. He found that aberrant chromosome constitution resulted in aberrant embryo development or cell death [Boveri, 1902, 1914]. From the simple organisms he studied, he inferred for tumor development that similar pathways are

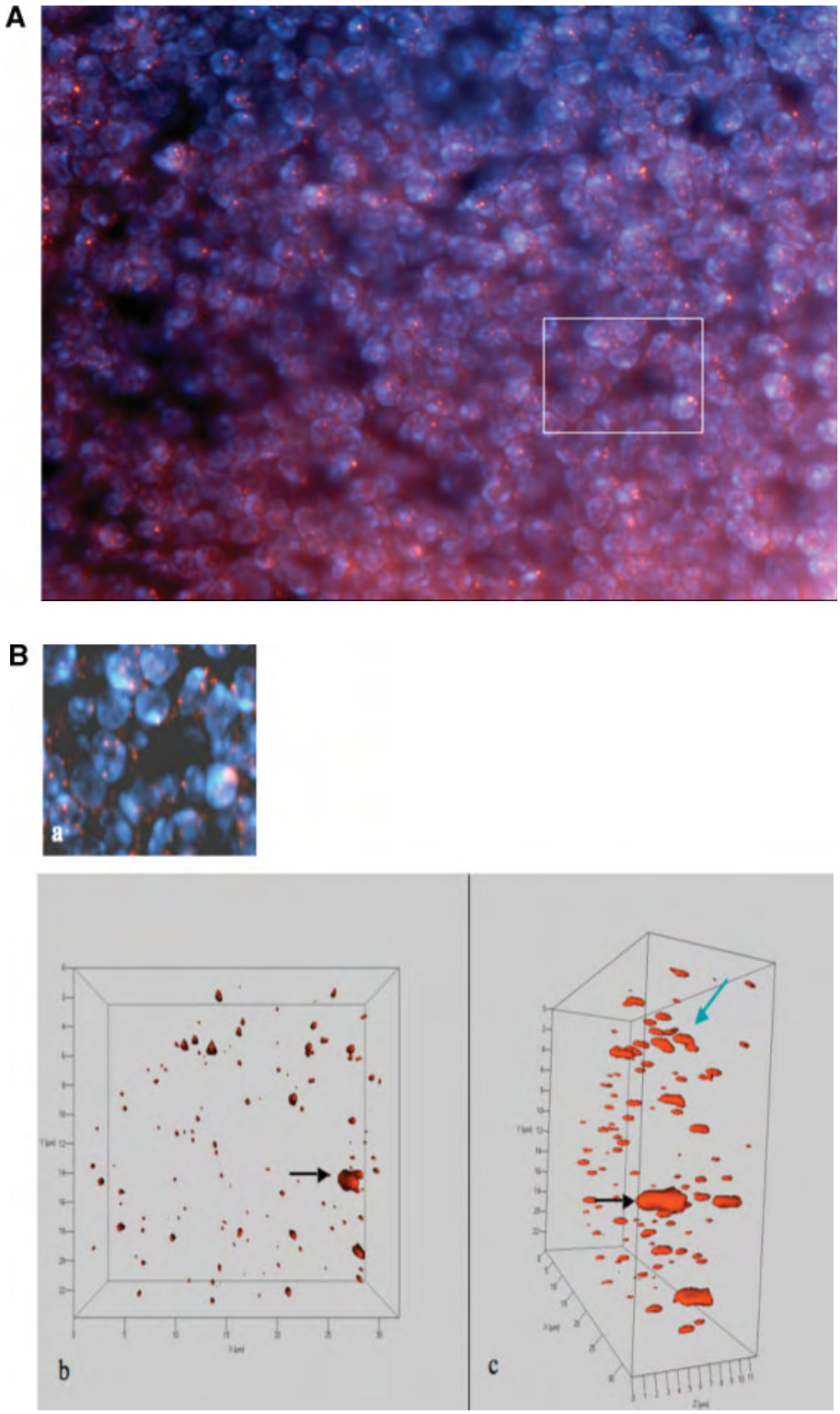

Fig. 4. Plasmacytotic focus examined by telomere hybridization. Telomere hybridizations were performed on 5-µm sections of paraffin-embedded tissues. **A**: Overview of plasmacytotic focus in 2D. Nuclei are shown in blue, telomeres in red. White box indicates area of the section that is shown in (**b**). **B**: Insert from (**a**) showing 2D and 3D organization of the telomeres. Black arrows point to TAs seen in front view (b) and in side view (**c**). The blue arrow points to a structure that appears to be a replicating telomere.

in operation [Boveri, 1914]. The centrosome cycles and aberrations thereof were also described by Boveri [1914] for the first time and later translated into English by his wife [Boveri, 1929]. Since his time, more details about the 3D organization of the nucleus and the genome have been investigated. However, the big picture that he first put forward is as valid today as it was in his time.

CURRENT CONCEPTS AND OPEN QUESTIONS

In the following paragraphs, we will discuss some of the issues that are important for future research in the area of the 3D organization of the nucleus and its alteration in the contribution to tumor development.

WHICH STIMULI LEAD TO THE FORMATION OF TAs?

Due to the impact of telomeric remodeling on genome stability, it will be important to characterize the conditions that lead to the formation of TAs. We have recently studied c-Myc-dependent TA formation and the effects of TAs on genomic instability [Louis et al., 2005]. We anticipate that other oncogenes may cause similar effects. One candidate is Ha-Ras. This oncogene was already studied with respect to its ability to alter chromatin organization [Fischer et al., 1998]. A more recent study using Balb/3T3 cells spontaneously immortalized and transfected with mutated c-Ha-Ras-1 found that Ha-Ras increased the level of chromosomal rearrangements involving telomeric sequences threefold [Peitl et al., 2002]. However, it is not known whether these rearrangements followed TA formation. It is also not known whether additional genetic changes occurred in these immortalized cells that contributed to the above results.

Other stimuli that converge at the chromosomal ends and elicit genomic instability may involve viruses that are able to immortalize or transform the host cells. Wan et al. [1997] examined telomeres after human papilloma viral infection and found a high frequency of telomeric associations and rearrangements. Using human ovarian epithelial cells immortalized by human papilloma oncoproteins, E6 and E7, the authors observed that 30–100% of all metaphases examined displayed telomeric associations (ibid). Whether these associations followed TA formation has not been investigated. However, one may postulate that this is very likely if the c-Myc-mediated remodeling of telomeres and chromosomes can be considered a general pathway to nuclear remodeling of the genome. The impact of viruses on telomeric organization and genomic instability requires further investigation. The above effects may be cell-type, host, and/or virus-specific since work by Argilla et al. [2004] demonstrates that transgenic mice expressing SV40 or HPV16 in the absence of telomerase do not exhibit telomere dysfunction or increased genomic instability.

TELOMERES AND EVOLUTION

It has been reported that human subtelomeric sequences are recombination and duplication hot spots [Linardopoulou et al., 2005]. Subtelomeric sequences are involved in interchromosomal recombinations and segmental duplications. This not only is a feature of tumor cells, but also occurs frequently during evolution. For example, half of the known subtelomeric sequences have formed recently during primate evolution. Interestingly, the subtelomeric gene duplication rate is significantly higher than the genome average. Thus, the authors conclude that this is both advantageous for evolution and may also have pathological consequences [Linardopoulou et al., 2005]. In the context of our discussion, we emphasize that telomeres and subtelomeric sequences are hot spots of evolution and genomic instability. The formation of TAs may contribute to both.

DO TAs AFFECT THE CHROMOSOMAL ORDER IN VIVO?

Since previous studies were done in established tumors or tumor cell lines [Chuang et al., 2004], one cannot say whether the chromosomal order changed due to tumor formation or due to TA formation or both. While data are emerging that TAs occur early in tumor development (Figs. 3 and 4), it is still unclear whether this is mechanistically linked to the remodeling of the nuclear order of chromosomes and to rearrangements in vivo. Thus, the cause-relationship in vivo is not yet established and needs to be examined carefully.

The closest cause-relationship study to date involved oncogenic remodeling of the telomeres and chromosomes in the nucleus [Louis et al.,

2005]. In this in vitro study using mouse lymphocytes, c-Myc deregulation led to TA formation that preceded chromosomal rearrangements via BBF cycles [Louis et al., 2005; Mai and Garini, 2005].

WHEN IS THE EARLIEST TIME POINT FOR TA FORMATION AND WHEN IS THE TUMORIGENIC POTENTIAL ESTABLISHED?

This question is critical for our understanding of the impact of nuclear remodeling in tumor development. We speculate that TAs formation may be the earliest event in tumor development and occur subsequent to oncogene deregulation. This is solely based on our in vitro studies and on studies we performed with pre-neoplastic and non-invasive lesions. More detailed studies in several tumor models will be necessary to establish this point. Is it enough for a cell to carry TAs to be tumorigenic? Are TAs and chromosomal rearrangements required before a cell becomes tumorigenic? Is a specific genetic background more susceptible to TA formation? Is the formation of TAs reversible? Can cells repair TAs? When do TAs become irreversible? Appropriate cell culture and mouse models will allow researchers to address such questions in the future.

WILL CELLS UNDERGO APOPTOSIS WHEN A CRITICAL THRESHOLD OF TAs IS REACHED?

Data on repeated c-Myc inductions suggest this may be the case. When mouse Pre B lymphocytes are stimulated to overexpress c-Myc every 12 h, >96% of all nuclei display large or several TAs. In this experimental set-up, all cells die of apoptosis within 30 h [Louis et al., 2005]. In contrast, a single activation of c-Myc deregulation for 2 h or for 12 h led to the formation of three or five TA cycles, respectively, which represent BBF cycles, without significant elevation in cell death (ibid). Thus, we propose that a critical threshold of TAs is tolerated by the cells and leads to genomic instability through chromosome remodeling by TA-induced BBF cycles. A low level of TAs allows for cell survival and cell proliferation while genomic rearrangements can occur. The latter situation is the critical one, since it contributes to the propagation of genomically unstable cells.

REMODELING OF THE NUCLEUS THROUGH TAs

In c-Myc deregulated cells, TAs form and chromosomes change their positions. Not only are there more chromosomal overlaps, but also more chromosomal ends become linked through TAs and fusions [Louis et al., 2005]. Several questions arise from these findings. Do chromosomes move normally? This is an open question, since the available data do not allow for a consensus in interpretation. While some research groups do not find substantial chromosomal movements [Abney et al., 1997; Gerlich et al., 2003], others find chromosomal reorganization during the cell cycle [Ferguson and Ward, 1992; Vourc'h et al., 1993; Bridger et al., 2000; Chubb et al., 2002; Walter et al., 2003; Essers et al., 2005], cellular differentiation [Stadler et al., 2004], and during quiescence and senescence [Bridger et al., 2000].

Whether chromosomes move normally or not, there are conditions that induce movement, such as c-Myc deregulation [Louis et al., 2005]. In the presence or absence of pre-existing movements, the potentially dynamic nature of chromosome order is a very complex issue. For example, are there specific neighborhood relationships that become established due to specific stimuli (such as oncogenic activation, viral infection, DNA damage)? Or do chromosomes that are observed in specific chromosomal neighborhoods come closer to each other diminishing the intrachromosomal space? Would this favor fusions, illegitimate recombinations, and/or non-homologous end joining?

There is evidence that a non-random nuclear order of chromosomes with specific chromosomal neighborhood relationships is important for specific rearrangements. Data by Neves et al. [1999] suggest this for *bcr/abl* in chronic myeloid leukemia. Chromosomes 9 and 22 are in close enough proximity to permit this translocation. This finding is supported by Kozubek et al. [1999] who state that the positions of chromosomes 9 and 22 have a determinative role in the induction of t(9;22) and in the development of t(9;22) leukemias. For mouse B cells, chromosomes 12 and 15 are found in a close neighborhood in lymphocytes (where they are involved in balanced translocations in mouse plasmacytoma) but are found more distant in mouse hepatocytes [Parada et al., 2004]. There are more studies that support this

chromosome neighborhood concept. Thomas and Diehl [2003] state that the proximity between translocating chromosomes is a prerequisite for their rearrangement. Roix et al. [2003] support this interpretation.

In a survey of >11,000 constitutional translocations, Bickmore and Teague [2002] concluded that the frequency of constitutional translocations depended on three main factors, and these included the chromosome positions, chromosome sizes, and specific DNA sequences.

We conclude from the above that chromosome specific neighborhood relationships exist in a cell-type specific manner and are consistent with the resulting chromosomal translocations. However, the experimental proof for this concept is lacking. For example, if a chromosome involved in translocations was moved to a new nuclear position would it still be involved in the same translocations or not? Do approaching gene loci or gene loci in the same nuclear compartment contribute to possible illegitimate recombination events? To date, these questions remain unanswered and await future investigation.

It is now possible to view all chromosomes in a nucleus [Bolzer et al., 2005]. Such 3D localization of all chromosomes needs to be combined with 3D FISH studies to assess potential gene and chromosomal region-associated movements.

CONCLUSIONS

Further research is required to fully understand the complexity of nuclear organization in normal cells and during malignancy. Studies using various approaches are required to investigate the complexity of 3D nuclear space that is crucial for understanding genome organization and stability. Geneticists, evolutionary biologists, cancer researchers, cell biologists, program developers, physicists, mathematicians, and biostatisticians are all necessary in a multidisciplinary effort to understand and model the nuclear structure and its regulation in normal and tumor cells. Only when we fully understand who the key players are, will we be able to learn how to modulate them for patient-specific treatments.

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ORIGINAL ARTICLE

Telomeric aggregates and end-to-end chromosomal fusions require myc box II

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Telomeres of tumor cells form telomeric aggregates (TAs) within the three-dimensional (3D) interphase nucleus. Some of these TAs represent end-to-end chromosomal fusions and may subsequently initiate breakage–bridge–fusion cycles. Wild-type (wt) and myc box II mutant (mt) Myc induce different types of genomic instability when conditionally expressed in mouse proB cells (Ba/F3). Only wt Myc overexpressing Ba/F3 cells are capable of tumor formation in severe combined immunodeficient mice. In this study, we investigated whether telomere dysfunction leading to TA formation is linked to the genetic changes that permit wt c-Myc-dependent transformation of Ba/F3 cells. To this end, we examined the 3D organization of telomeres after the deregulated expression of deletion myc boxII mutant (Δ106) or wt Myc. Δ106-Myc overexpression did not induce TAs, whereas wt-Myc deregulation did. Instead, Δ106-Myc remodelled the 3D telomeric organization such that telomeres aligned in the center of the 3D interphase nucleus forming a telomeric disk owing to a Δ106-induced G1/S cell cycle arrest. In contrast, wt-Myc overexpression led to distorted telomere distribution and TA formation. Analysis of chromosomal alterations using spectral karyotyping confirmed Δ106-Myc and wt-Myc-associated genomic instability. A significant number of chromosomal end-to-end fusions indicative of telomere dysfunction were noted in wt-Myc-expressing cells only. This study suggests that TAs may play a fundamental role in Myc-induced tumorigenesis and provides a novel way to dissect tumor initiation.
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Introduction

Chromosomes are organized into discrete territories in the interphase nucleus. This nonrandom organization of chromosomes, conserved during evolution, suggests a role for a spatial organization in the control of gene expression and replication (for reviews, see Cremer and Cremer, 2001; Parada *et al*., 2002). A number of reports describe an architectural stability of the chromosomal positions in the nucleus (Gerlich *et al*., 2003), whereas other studies described considerable changes in chromosomal positions during the cell cycle (Gasser, 2002; Essers *et al*., 2005). In particular, chromosome motility increases during mitosis and early-stage G1, but it is limited to local diffusion during the rest of cell cycle (Walter *et al*., 2003). The spatial organization of chromosomes might contribute to chromosomal translocations found in many tumors (Kozubek *et al*., 1999; Neves *et al*., 1999).

Three-dimensional (3D) fluorescent *in situ* hybridization (FISH) is an innovative approach to study the nuclear architecture in fixed cultured cells during cell cycle, cell differentiation and malignant transformation (Solovei *et al*., 2002; Cremer *et al*., 2003).

Using 3D FISH experiments with peptide-nucleic-acid (PNA)-telomeric probes, we demonstrated that telomeres of normal cells are organized in a nonoverlapping manner in the 3D interphase nucleus (Chuang *et al*., 2004; Louis *et al*., 2005). In contrast, tumor cells display an aberrant organization of telomeres and form clusters of telomeres, the so-called telomeric aggregates (TAs) (Chuang *et al*., 2004; Mai and Garini, 2005, 2006).

The position of telomeres during the cell cycle is an important indicator of the stage at which these fusions may occur. It has been shown previously that the 3D telomere organization varies during different phases of the cell cycle and displays a highly ordered, dynamic assembly in the interphase nucleus. During G0/G1 and S phases, telomeres are widely distributed throughout the nucleus, whereas in late G2 phase, they align in the middle of the nucleus forming a telomeric disk (Chuang *et al*., 2004; Vermolen *et al*., 2005).

The deregulation of Myc protein is found in a wide range of human cancers and is associated with disease progression. The deregulated expression of Myc can

drive cells into proliferation (Deb-Basu *et al*., 2006), reduce cell adhesion (Frye *et al*., 2003), promote metastasis (Pelengaris *et al*., 2002) and genomic instability (for a review see Mai and Mushinski 2003; Kuttler and Mai, 2005).

The N-terminus of Myc has three highly conserved elements, known as Myc boxes. Of these, Myc box I has been implicated in Myc turnover (Bahram *et al*., 2000). Myc box I is essential for Myc function *in vivo* and is required for full transactivation and repression of many target genes (Oster *et al*., 2003). Myc box II is required for all the known biological functions of Myc (Stone *et al*., 1987) but not all Myc target genes require the integrity of this box for activation, which shows that there are other mechanisms of Myc-dependent activation (Nikiforov *et al*., 2002)

The conditional expression of wild-type (wt) Myc and deleted box II mutant-Myc (Δ106-Myc) in spontaneously immortalized mouse Ba/F3 pro-lymphocytes was previously characterized (Fest *et al*., 2002). Mutant Myc protein induced lower level of apoptosis but higher level of genomic instability than its wt counterpart. It is of note that in these cells, genomic instability and tumorigenesis are two separable events: Only wt-Myc but not Δ106-Myc-expressing cells induced tumor formation in the severe combined immunodeficient mouse model (Fest *et al*., 2005).

We have recently reported that c-Myc deregulation induces cycles of TA formation and remodels the interphase nucleus by changing the organization of telomeres and chromosomes (Louis *et al*., 2005, for reviews, see Mai and Garini, 2005, 2006). In addition, the presence of TAs in cells constitutively expressing Myc contributed to genomic instability by forcing abnormal chromosome segregation during mitosis (Ermler *et al*., 2004).

Telomere function is essential for the preservation of chromosomal integrity (for a review see Feldser *et al*., 2003). Loss of various telomere-capping proteins or critical shortening of the telomeric repeats led to dysfunctional telomeres. The formation of dicentric chromosomes that led to specific rearrangements was observed more than 60 years ago by Barbara McClintock. Dicentric chromosomes can initiate ongoing chromosomal instability via breakage–bridge–fusion (BBF) cycles (McClintock, 1941). During mitotic segregation, the two centromeres of a dicentric chromosome are pulled to opposite poles and chromosomes can break. These breaks generate telomere-free ends and new chromosome fusions, nonreciprocal translocations and overall genetic changes that contribute to genomic instability. Our previous study showed that c-Myc is one key factor that initiates chromosomal rearrangements through BBF cycles (Louis *et al*., 2005).

In the present study, we investigated whether Myc box II is required for TA formation in Ba/F3 cells. In order to evaluate the difference in initiating and promoting tumorigenesis between wt-Myc and Δ106-Myc-expressing cells, we analysed the organization of telomeres in the interphase nucleus and the presence of chromosomal rearrangements resulting from BBF cycles.

Results

Telomere disk after cell cycle synchronization

It has been shown previously that varying telomere organization is observed during different phases of the cell cycle with telomeric disks forming in the G2 phase of the cell cycle (Chuang *et al*. 2004). Telomere positions in the 3D nucleus were calculated by using a program (Teloview) and algorithms that we have developed for this purpose (Chuang *et al*. 2004; Vermolen *et al*., 2005). Briefly, using an adequate threshold, the program calculates the center of gravity, the volume and intensity for each telomere. Using the quick-hull algorithm (Barber *et al*. 1996), the distribution of the telomeres in the nucleus volume is found by fitting the smallest set of polygons that contains all the telomeres. In general, this volume is an ellipsoid with two similar radii ($a=b$) and one dissimilar radius (c) (i.e. spheroid). Therefore, the level of flatness of the volume occupied by the telomeres can be described by an a/c ratio. The larger the ratio, the more disk-like is the shape of the volume occupied by the telomeres.

In order to confirm the position of telomeres during different phases of the cell cycle and the presence of telomeric disk, mouse diploid immortalized Pre-B lymphocytes were synchronized in late G2 with 0.5 μg/ml of nocodazole. Synchronized cells were reintroduced into culture and harvested again after 8 h at G1 phase (Figure 1a).

Using PNA-FISH hybridization in 3D fixed Pre-B lymphocytes, we confirmed the formation of a telomeric disk at the time of synchronization (Figure 2b). Eight hours after release from synchronization, telomeres returned to a wide distribution throughout the interphase nucleus (Figure 2c). The calculated a/c ratios in the cells arrested in G2/M phase and in the cells in G1 phase were 11.8 ± 2.9 and 8.1 ± 1.7, respectively (Table 1).

To detect telomere positions after G1/S synchronization, Pre-B cells were incubated for 42 h in RPMI 1640 that had been depleted of the amino acids methionine, cysteine and L-glutamine, and then returned to complete RPMI 1640 with mimosine at a concentration of 0.4 μg/ml 8 h (Kuschak *et al*., 2002). The G1/S block was confirmed by fluorescent-activated cell sorter (FACS) analysis (Figure 1a). Eight hours after release from the G1/S block, Pre-B cells returned to normal cycling conditions (Figure 1b). Under conditions of G1/S synchronization, the telomeres aligned in the center of 3D interphase nucleus and formed a telomere disk (Figure 2d). The high value of the a/c ratio (9.6 ± 2.9 time 0), calculated for G1/S synchronized cells, confirmed the flatness of the volume occupied by the telomeres. Eight hours after release from the G1/S arrest, the telomeres were widely distributed in 3D interphase nucleus with a calculated a/c ratio of 7.7 ± 1.4 (Table 2, Figure 2e).

The 3D organization of telomeres in wt-Myc and Δ106-Myc-induced Ba/F3 cells

To study the organization of telomeres in the nucleus after conditional wt-Myc and Δ106-Myc induction in

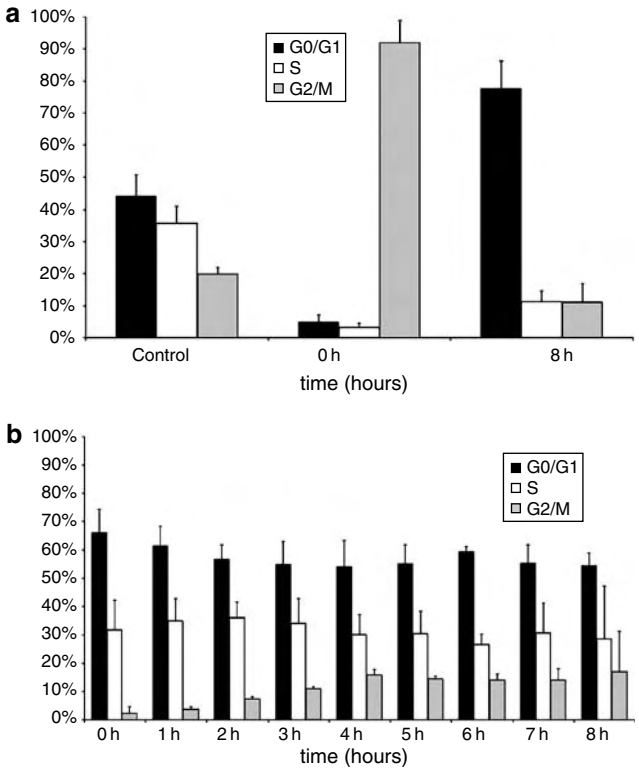

Figure 1 FACS analysis for Pre-B cells. Control group proliferated normally with no detectable sign of cell cycle arrest (**a**). Cells were harvested 0 h after having been synchronized at G2/M with nocodazole and 8 h after release from synchronization with nocodazole. (**b**) For G1/S synchronization, Pre-B cells were incubated for 42 h in RPMI media that had been depleted of the amino acids methionine, cysteine and L-glutamine, released from G1/S synchronization and placed in normal media. Cells were then harvested at 0 h after synchronization and once every hour for 8 h. The cell cycle profile was expressed as the percentage (±s.d.) of cells in each phase (G0/G1; S; G2/M). These values were calculated from data collected from three independent experiments.

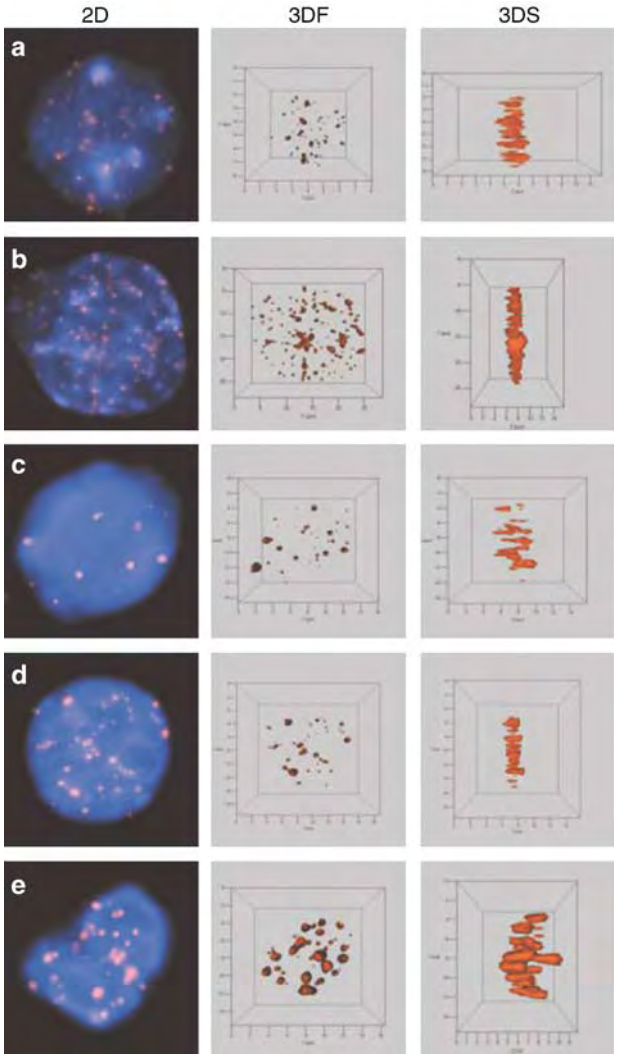

Figure 2 Three-dimensional analysis of telomere position after G2/M and G1/S synchronization. (**a**) Fixed control cell untreated. (**b**) Fixed cells harvested 0 h after having been synchronized at G2/M with nocodazole. (**c**) Fixed cells harvested 8 h after release from synchronization with nocodazole. (**d**) Fixed cells harvested 0 h after synchronization at G1/S with depleted medium/mimosine. (**e**) Fixed cells harvested 8 h after release from synchronization with depleted medium/mimosine. Telomeres are shown in red; nuclei were stained with DAPI (blue). 3DF (3D front view), 3DS (3D side view).

immortalized mouse pro-B lymphocytes (Ba/F3) stably transfected with MycER (Fest *et al*., 2002), we performed PNA-telomere FISH hybridization. After addition of a single dose of 4-hydroxytamoxifen (4HT), nuclear c-Myc signal was quantified by quantitative fluorescent immunostaining (Kuschak *et al*., 1999). In nontreated control cells, MycER was found in the cytoplasm. The nuclear signal of both wt and Δ106-Myc proteins increased threefold over a 2–4-h period and decreased to the levels of nontreated cells after 6 h (data not shown).

Consistent with our previous results (Louis *et al*., 2005), Ba/F3 cells without MycER activation showed nonoverlapping telomere positions (Figure 3a). At 24 h after wt-Myc activation, Ba/F3 cells displayed a wide spatial telomere distribution and the presence of TAs (Figure 3b). At the same time point, in Δ106-myc-induced Ba/F3 cells, telomeres were aligned in the center of the 3D interphase nucleus (Figure 3c).

To better describe telomere distribution in 3D nucleus, we measured *a*/*c* ratios after wt and Δ106-Myc activation (Table 3). Telomeres were widely

Table 1 Effect of G2/M synchronization on telomeres distribution in 3D nucleus

Cell culture conditions	*a*/*c ratio*	*Telomere distribution in 3D*
Pre-B control	6.3±2.3	Widely distributed throughout the nucleus
Pre-B 0 h + nocodazole	11.8±2.9	Disk formation
Pre-B 8 h − nocodazole	8.1±1.7	Widely distributed throughout the nucleus

3D, three-dimensional. Thirty nuclei were analysed for each time point. These values were calculated from data collected from three independent experiments. Values are means of three experiments±s.d.

Table 2 Effect of G1/S synchronization on telomeres distribution in 3D nucleus

Cell culture conditions	*a/c ratio*	*Telomere distribution in 3D*
Pre-B 0 h (amino-acid deprivation plus mimosine)	9.6±2.9	Disk formation
Pre-B 8 h after release from G1/S block	7.7±1.4	Widely distributed throughout the nucleus

Thirty nuclei were analysed for each time point. These values were calculated from data collected from three independent experiments. Values are means of three experiments±s.d. 3D, three-dimensional.

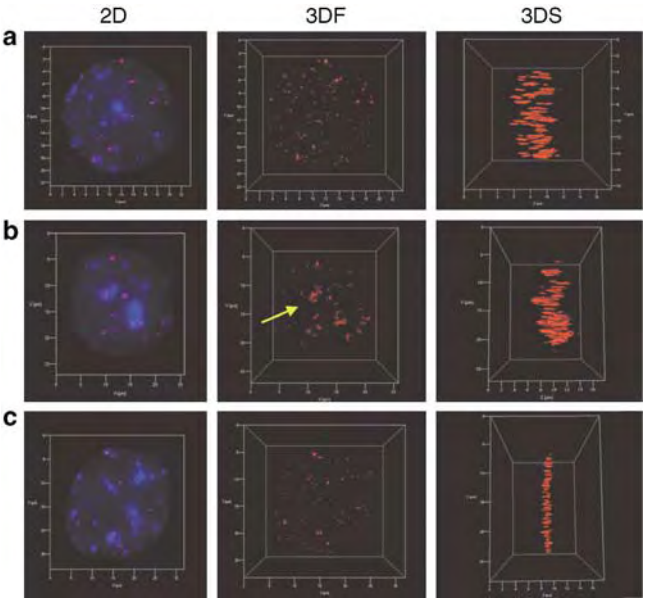

Figure 3 Telomere distribution in 3D interphase nuclei of Ba/F3 cells at 24 h after wt-Myc and Δ106-Myc activation. (**a**) Control (non-4HT treated) Ba/F3 cells; (**b**) wt-Myc-activated Ba/F3 cells with a wide distribution of telomeres and with TAs formation (yellow arrow); (**c**) in Δ106-Myc expressing cells, telomeres are aligned in the center of the interphase nucleus, forming a disk-like structure without TAs. Telomeres are shown in red; nuclei were stained with DAPI (blue). 3DF (3D front view), 3DS (3D side view).

distributed throughout the nucleus after wt-Myc induction with a calculated *a/c* ratio of 4.1±1.1 at 24 h and 4.9±0.7 at 48 h, which means a spherical-like volume of distribution. However, after Δ106-Myc induction, the *a/c* ratio was 10.1±4.3 at 24 h and 9.2±2.9 at 48 h.

The possible relationship between telomere distribution and cell cycle in this model was investigated by flow cytometry. Propidium iodide staining of cellular DNA indicated that non-4HT-treated Ba/F3 cells proliferated normally with no detectable sign of cell cycle arrest (Figure 4, 0 h). As expected, the overexpression of wt-Myc increased G1/S transition after 24 and 48 h (Figure 4a). In contrast, the Δ106-Myc-expressing cells accumulated at G1/S phase of the cell cycle. By 24 h, over 80% of the cells were arrested in G1/S (Figure 4b).

Table 3 Effect of wt-Myc and Δ106-Myc expression on telomeres distribution in 3D nucleus

Cell culture conditions in the presence of 4HT	*a/c ratio*	*Telomere distribution in 3D*
BaF3 wt-Myc 0 h	6.7±1.3	Widely distributed throughout the nucleus
BaF3 wt-Myc 24 h	4.1±1.1	Widely distributed throughout the nucleus
BaF3 wt-Myc 48 h	4.9±0.7	Widely distributed throughout the nucleus
BaF3 Δ106-Myc 0 h	6.7±1.1	Widely distributed throughout the nucleus
BaF3 Δ106-Myc 24 h	10.1±4.3	Disk formation
BaF3 Δ106-Myc 48 h	9.2±2.9	Disk formation

Thirty nuclei were analysed for each time point. These values were calculated from data collected from three independent experiments. Values are means of three experiments±s.d. 3D, three-dimensional.

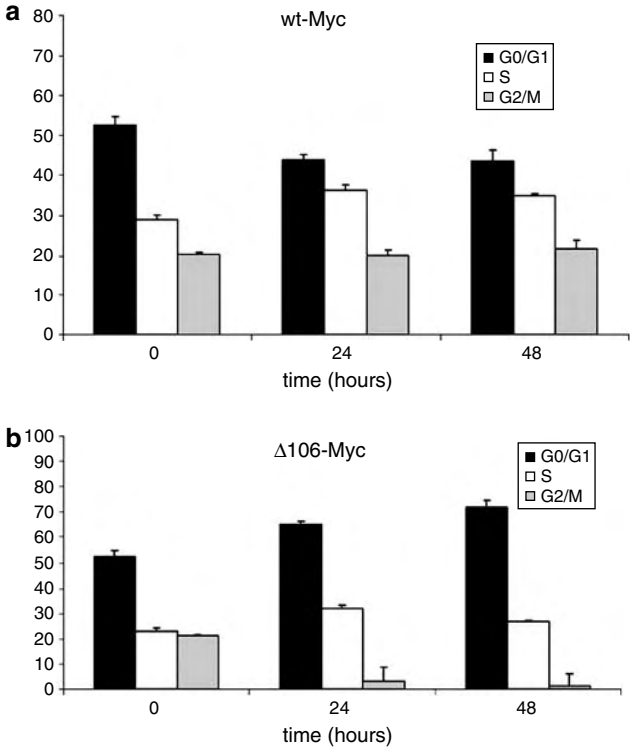

Figure 4 Effect of wt-Myc and Δ106-Myc expression on cell cycle profiles. Cell cycle analysis of Ba/F3 cells at 24 and 48 h after wt-MycER (**a**) and Δ106-MycER (**b**) activation. The cells were collected, permeablized and DNA was stained with propidium iodide. The overexpression of wt-Myc increased G1/S transition after 24 and 48 h. The Δ106-Myc-expressing cells accumulated at G1/S phase of the cell cycle. The cell cycle profile was expressed as the percentage (±s.d.) of cells in each phase (G0/G1, S, G2/M). These values were calculated from data collected from three independent experiments.

Δ106-Myc expression does not induce TA formation in interphase nuclei

In tumor cells, the ordered and nonoverlapping 3D nuclear space that telomeres normally occupy is compromised and telomeres can form aggregates that

may fuse their respective chromosomes, favoring structural chromosomal aberrations. In order to investigate whether the mutation in Myc box II impacted on the formation of TAs, we analysed wt and Δ106-Myc-expressing cells. To this end, cells were harvested every 6 h over a time period of 48 h. Measurement of TAs was performed after 3D image acquisition and constrained iterative deconvolution (Louis *et al*., 2005).

This time course experiment confirmed that only wt-Myc expression in Ba/F3 cells induced TAs. Representative images showed that TAs varied in number in wt-Myc-expressing cells at 24 h (Figure 5B, b). In contrast, TAs were not detectable in the 3D nucleus at 24 h, with control Ba/F3 cells (non-4HT treated cells) and Δ106-Myc-expressing cells (Figure 5B, a and c, respectively). A single dose of 4HT induced the highest levels of TA formation after 24 h in wt-Myc-expressing cells. Thus, only a single TA cycle is observed in Ba/F3 after wt-Myc activation (Figure 5A). In Ba/F3 cells, the nuclear localization of Myc completely disappeared 6 h after 4HT-induced MycER activation (data not shown). As reported previously (Louis *et al*., 2005), the number of TA cycles was directly linked to the duration of wt-Myc deregulation. As wt-Myc but not Δ106-Myc-expressing cells induced tumorigenesis *in vivo* (Fest *et al*., 2005), the presence of TAs seems to be linked to the initiation and/or progression of tumorigenic potential seen in Ba/F3 cells with deregulated wt-Myc expression.

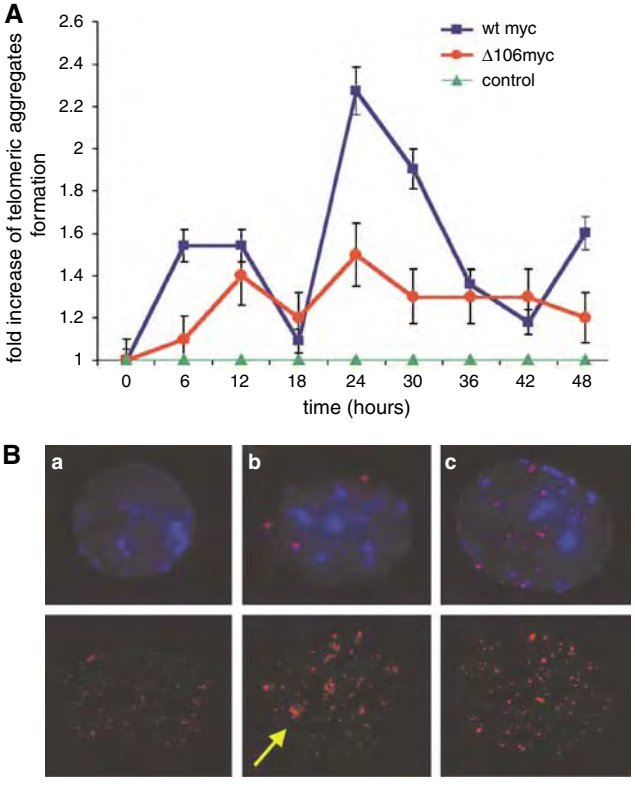

Figure 5 Overview of TA formation in nuclei of Ba/F3 cells expressing wt-Myc or Δ106-Myc. (**A**) Fold increase in TAs over control level during a period of 48 h. The highest levels of TAs were observed at 24 after wt-Myc activation. Error bars represent a 95% confidence interval of binomial distribution. (**B**) Representative individual images showing TA formation over the time frame shown in (**A**). (**B**, a) Control (non-4HT treated) Ba/F3 cells display nonoverlapping telomeres. (**B**, b) wt-Myc activated Ba/F3 show the formation of TAs (yellow arrow). (**B**, c) Δ106-Myc-expressing cells do not show TAs.

A significant number of chromosomal fusions were noted only in wt-Myc-expressing cells

To determine whether the formation of TA was associated with BBF events, spectral karyotyping (SKY) analysis on metaphase chromosomes was performed at different times: prior (6 h), during (24 h) and after (42 h) the peak of TA formation in both wt and Δ106-Myc-expressing cells. Table 4 summarizes the genomic aberrations detected in wt-Myc-expressing Ba/F3 cells. As expected for an immortalized cell line (Fest *et al*., 2005), control Ba/F3 cells (non-4HT treated)

Table 4 Chromosomal rearrangements in wt-Myc-activated Ba/F3 cells

Time point (h)	*Aberrations*	*%*	P-*value*
0	Fusions: none Translocation: T5;X, T4;9 Metaphase with translocations: 2/20 Average number of translocations per metaphase: 1 Metaphases with chromosomal fusions: 0/20 Fusions per metaphase: 0	10 —	0.1468 —
6	Fusions: none Translocation: T4;9, T5;X, T14;X Metaphase with translocations: 3/20 Average number of translocations per metaphase: 3 Metaphases with chromosomal fusions: 0/20 Fusions per metaphase: 0	15 —	0.0717 —
24	Fusions: 8:19, 14:11, X:X, 10:9, 19:X, 18 :18, 11:6 Translocation: T15;9, T15;2, T5;X, T15; 10 Metaphase with translocations: 6/20 Average number of translocations per metaphase: 5 Metaphases with chromosomal fusions: 7/20 Fusions per metaphase: 3	30 35	0.0101 0.0036
42	Fusions: 10:11, X:X Translocation: T5;X, T8;4, T4;10, T15;10 Metaphase with translocations: 2/20 Average number of translocations per metaphase: 3 Metaphases with chromosomal fusions: 6/20 Fusions per metaphase: 2	10 30	0.1468 0.0101

The table summarizes data obtained by spectral karyotyping. Metaphases were prepared and analysed after a single administration of 4-hydroxytamoxifen to wt-MycER Ba/F3 cells (Figure 3). End-to-end fusions were detected at 24 h in 30% of the metaphases ($P = 0.0101$). Nonreciprocal translocation were also found at 24 h (in 35% of the metaphases) and reached significance. As time progresses, fusions decreased whereas translocations remained steady. The table shows the description of the aberrations found, their percentage of occurrence, average number of fusion and translocation per metaphase, and significance *P*-values. A minimum of 20 metaphases was examined per time point.

showed some chromosomal alterations. At 24 h, six out of 20 metaphases in wt-Myc-expressing cells showed a significant increase in end-to-end chromosomal fusions and nonreciprocal translocations over control levels. At 42 h after wt-Myc activation, the percentage of fusions and translocation decreased. The karyotype of spontaneously immortalized, tetraploid (Ba/F3) cells is shown in Figure 6a. Representative images (Figure 6b), 24 h after wt-Myc activation, show fusions at telomeric ends between two chromosomes X and between chromosomes 10 and 9. Nonreciprocal translocation between chromosomes 15 and 9 and broken chromosomes (4, 16 and 9) were observed. Other aberrations included the insertion of chromosome X material into chromosome 1 and of chromosome 19 material into chromosome 4.

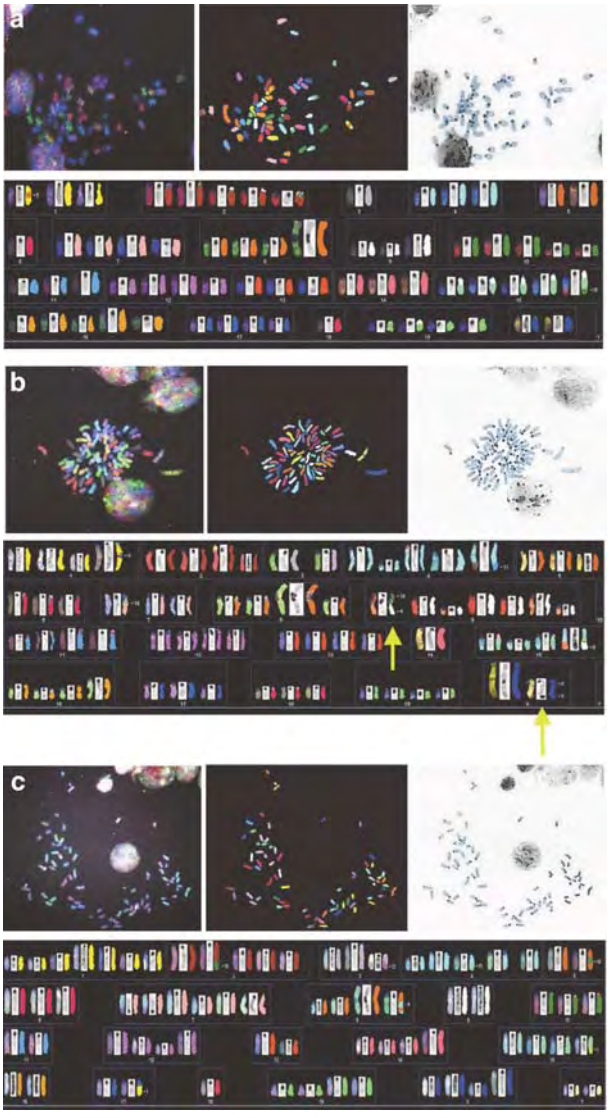

Figure 6 SKY of Ba/F3 wt-MycER and Δ106-MycER cells. Representative images of Ba/F3 cells control (non-4HT treated) (**a**), 24 h after MycER activation (+4HT) for (**b**) wt-Myc and Δ106-Myc (**c**) are shown. Notice the presence of fusion between two chromosomes X and between chromosome 10 and 9 (see yellow arrows) after wt-Myc activation. No fusions were detected by SKY after Δ106-Myc activation. For a detailed overview of the aberration detected by SKY, see Tables 4 (wt-Myc) and 5 (Δ106-Myc). Each panel of the figure shows the following order of images, the raw spectral image of the metaphase (top left corner), the classified image of the identical metaphase (top middle panel), the inverted DAPI image of the identical metaphase (top right corner) and the classified karyotype of the identical metaphase is displayed in the bottom panel. A minimum of 20 metaphases was analysed per time point.

The analysis of 20 metaphases from Δ106-Myc-expressing cells revealed an increased number of nonreciprocal translocations over 42 h in comparison with control (non-4HT treated) Ba/F3 cells. In contrast to wt-Myc-overexpressing Ba/F3 cells, Δ106-Myc-overexpressing cells did not show chromosomal end-to-end fusions. Nonreciprocal translocations were found in wt-Myc-expressing cells at 24 h in 30% of the metaphases ($P = 0.0101$). As time progressed, translocations increased (in 40% of metaphases $P < 0.001$) (Table 5). Figure 6c summarizes the most common aberrations seen in Δ106-Myc-expressing cells at 24 h. SKY analysis confirmed the presence of unbalanced translocation involving chromosomes 2, 3, 4 and 15.

Both wt and Δ106-Myc proteins showed high levels of significant karyotypic instability. The main difference between Δ106-Myc and wt-Myc-expressing cells were that the latter exhibited a significant number of chromosome fusions related to telomere dysfunction.

Discussion

There are at least two types of telomere dysfunction in tumor cells. One type involves critically short telomeres (DePinho and Polyak, 2004). The other one involves the formation of TAs and is independent of telomere size and telomerase activity (Louis *et al*., 2005). Telomeres of normal cells are organized in a nonoverlapping manner in the 3D interphase nucleus. In contrast, tumor cells display an aberrant organization of telomeres that impact on the numbers of TAs (Chuang *et al*., 2004).

Telomere dynamics have been observed and measured by live cell imaging approaches in different cell lines. Long and short ranging movements were observed over a time period of 20 min (Molenaar *et al*., 2003). 3D imaging has permitted to determine that the telomere organization in the nucleus is cell cycle dependent. As such the position of telomeres during the cell cycle is an important indicator of the stage at which these TAs may occur (Chuang *et al*., 2004).

In the present study, we have investigated a possible correlation between telomere positions during the cell cycle and the formation of TAs and the different tumorigenic potential of wt and mutant Myc proteins (Fest *et al*., 2005). At the G1/S boundary of a synchronized cell cycle, the telomeres of Pre-B cells have a tendency to align in the center of the nucleus, in a structure we had termed earlier a telomeric disk (Chuang *et al*., 2004). This is the first time such a telomere organization has been found during an induced G1/S block. This alignment of telomeres dissociates and telomeres are observed throughout the nucleus when cells re-enter into the cycle after release from the synchronization event.

Table 5 Chromosomal rearrangements in Δ106-Myc activated Ba/F3 cells

Time point (h)	*Aberrations*	*%*	P-*value*
0	Fusions: none Translocation: T5;X Metaphase with translocations: 2/20 Average number of translocations per metaphase: 1 Metaphases with chromosomal fusions: 0/20 Fusions per metaphase: 0	10 —	0.1468 —
6	Fusions: none Translocation: T5 ;1, T3 ;16, T 7 ;13 Metaphase with translocations: 3/20 Average number of translocations per metaphase: 3 Metaphases with chromosomal fusions: 0/20 Fusions per metaphase: 0	15 —	0.0717 —
24	Fusions: none Translocation: T5;X, T1;15, T15;4, T12;3, T2;10 Metaphase with translocations: 6/20 Average number of translocations per metaphase: 4 Metaphases with chromosomal fusions: 0/20 Fusions per metaphase: 0	 30 —	 0.0101 —
42	Fusions: none Translocation: T15;4, T7;13, T8;4, T14;X Average number of translocations per metaphase: 8/20 Translocations per metaphase: 4 Metaphases with chromosomal fusions: 0/20 Fusions per metaphase: 0	 40 —	 <0.001 —

The table summarizes data obtained by spectral karyotyping. Metaphases were prepared and analysed after a single administration of 4-hydroxytamoxifen at Δ106-MycER Ba/F3 cells (Figure 3b). End-to-end fusions were not observed over the 42 h of the experiment. Nonreciprocal translocations were found at 24 h in 30% of the metaphases ($P=0.0101$). As time progressed, translocations increased (in 40% of metaphases $P<0.001$). The table shows the description of the aberrations found, their percentage of occurrence, average number of fusion and translocation per metaphase, and significance *P*-values. M, metaphase. A minimum of 20 metaphases was examined per time point.

The telomeric disk naturally reforms at the G2/M transition of the cell cycle (Chuang *et al*., 2004). Thereafter, the telomeres resume their distribution throughout the nucleus. This feature of a dynamic telomeric organization throughout the cell cycle is mimicked by an experimental G2/M synchronization. Eight hours after release from the G2/M block, telomeres will assume their normal cell cycle-dependent organization.

The expression of wt-Myc stimulates the G1/S transition by regulating the levels and the activity of the cyclins (Trumpp *et al*., 2001). In Ba/F3 cells, wt-Myc activation promotes G1/S transition and is accompanied by a distorted telomere distribution that results from the presence of TAs (Figure 3, yellow arrow). In contrast, Myc box II mutants expressing cells were blocked in G1/S and the telomeres were aligned in the center of the 3D interphase nucleus, forming a telomere disk. These results agree with the data obtained in synchronized Pre-B cells in G1/S phase of the cell cycle.

Previous work had shown in Ba/F3 cells that wt and mutant Myc proteins induced genomic instability but only wt-Myc protein had the potential of initiating and promoting tumorigenesis *in vivo* (Fest *et al*., 2005). Using PNA-telomere FISH hybridization in 3D-fixed cells, we demonstrated that the formation of TAs takes part in MYC-induced tumorigenesis.

TAs were detectable only in wt-Myc expressing and tumorigenic Ba/F3 cells (Fest *et al*., 2005) reaching the highest peak after 24 h after Myc activation, whereas Δ106-Myc expressing and nontumorigenic Ba/F3 cells (Fest *et al*., 2005) did not show a significant number of TAs. The presence of TAs in malignant cells is supported by data from different cell lines and human tumors (Chuang *et al*., 2004).

SKY data show that conditional wt-Myc protein expression (Table 4) led to a higher level of chromosomes end-to-end fusions than conditional Δ106-Myc protein expression (Table 5). These results are in agreement with the absence of TAs in the mutant Myc-expressing cells.

TAs are not just a transient aberration in the 3D organization of the nucleus, but these events precede the formation of BBF cycles. As reported in our previous work, once aggregates form and chromosome fusions occur, BBF cycles result and the genetic information of the chromosomes will be remodelled (Louis *et al*., 2005).

Nothing is known about the mechanisms that cause TA formation in the context of c-Myc deregulation. Shelterin is a protein complex with DNA remodelling activity that, together with several DNA repair protein, such as WRN, the Mre1 complex and DNA-PK, protects the integrity of the chromosome ends (De Lange, 2005). In cells constitutively expressing Myc and characterized by the presence of TAs (Ermler *et al*., 2004), the level of TRF2 protein, a shelterin subunit protein, was reduced. These data lend support to the hypothesis that Myc may somehow interact with proteins of the shelterin or DNA repair complexes to mediate TAs formation. This capacity is lost in myc box II mutant. Understanding whether TAs may be the earliest events in tumor development and which genetic background is more susceptible to TA formation will provide a novel way to dissect the benign-to-malignant transition in cancer.

Materials and methods

Cell cultures and treatments

Mouse Pre-B lymphocytes (Mai *et al*., 1999) were grown in RPMI 1640 supplemented with 0.1% β-mercaptoethanol, 1% L-glutamine, 1% sodium-pyruvate, 1% penicillin/streptomycin and 10% fetal bovine serum (FBS; Gibco, Burlington ON, Canada) at 37°C, in a humidified atmosphere and in the presence of 5%CO_2.

For G2/M synchronization, Pre-B cells were incubated for 8 h in standard RPMI 1640 medium (Gibco, Burlington ON,

Canada) with nocodazole (Sigma-Aldrich, Oakville ON, Canada) at a concentration of 1 μg/ml. Upon completion of synchronization, cells were removed from nocodazole and returned to nocodazole-free media and harvested at 0 and 8 h.

For G1/S synchronization, Pre-B cells were incubated for 42 h in RPMI 1640 that had been depleted of the amino acids methionine, cysteine and L-glutamine, and then returned to complete RPMI 1640 with mimosine at a concentration of 0.4 μg/ml for 8 h (Kuschak *et al*., 2002). Cells were then harvested at 0 h and once every hour for 8 h.

The two Ba/F3 cell lines with conditional wt-MycER (Littlewood *et al*., 1995) and Δ106-MycER used in this study have been previously described (Fest *et al*., 2002). Cells were grown in RPMI 1640 containing 10% FBS, 1% WEHI cells supernatant (mouse myelomonocytic leukemia macrophage-like cells derived from a BALB/c mouse; the cells produce IL3 supernatant) and 0.21% of plasmocin (Cayla, Toulouse, France).

Wt-Myc and Δ106-Myc cells were induced with 4-hydroxytamoxifen (4HT) (Sigma-Aldrich, Oakville ON, Canada) to a final concentration of 100 nM in 10^5 cells/ml to activate the c-Myc protein. Cells were split 24 h prior induction and every 48 h after 4HT induction.

Cells were grown and maintained at a density of 10^5–10^6 cells/ml. Cell viability was determined by hemocytometer counts using trypan blue (Sigma-Aldrich, Oakville ON, Canada).

Immunohistochemistry

Immunohistochemistry was performed as described previously (Fukasawa *et al*., 1997). The primary antibody used was a rabbit polyclonal anti-c-Myc (N262) at a dilution of 1:100 (Santa Cruz, Santa Cruz, California, USA) visualized by using a secondary goat anti-rabbit IgG fluorescein isothiocyanate antibody at a dilution of 1:100 (Sigma-Aldrich, Oakville ON, Canada). Images were acquired using a Hamamatsu CCD SensiCam Camera and the Northern Eclipse v 6.0 software.

FACS analysis

For FACS analysis, Pre-B and Ba/F3 cells were fixed in 70% cold ethanol and stained with propidium iodide (Sigma-Aldrich, Oakville ON, Canada) (1 μg/ml) following RNAse digestion (Sigma-Aldrich, Oakville ON, Canada) (20 μg/ml). The stained cells were analysed for DNA content by flow cytometry in an EPICS Altra cytometer (Beckman-Coulter, Mississauga, ON, Canada).

Telomere FISH and 3D image analysis

Cells were fixed using 3:1 methanol/acetic acid fixative (Fluka, Oakville, ON, Canada) and then placed on $26 \times 76\,mm^2$ microscope slides. Telomeres were stained using quantitative fluorescent FISH with a telomere-specific CY3-labeled PNA probe (DAKO, Mississauga, ON, Canada). Nuclear volumes did not significantly change during the denaturation protocol used for 3D telomere FISH. Counterstaining was performed with 4′-6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, Oakville ON, Canada). Three independent experiments were performed and at least 30 nuclei were examined per time point.

Cells were imaged on a Zeiss Axioplan 2 microscope with a Zeiss Axiocam HRm and deconvolution module, including Axiovision (Zeiss, North York, ON, Canada) software v3.1. Images were deconvolved using a constrained iterative algorithm (Schaefer *et al*., 2001). Analysis was performed with TeloView (Chuang *et al*., 2004; Vermolen *et al*., 2005).

By choosing a simple threshold for the telomeres, the volume, intensity and center of gravity are calculated. The integrated intensity of each telomere is calculated because it is proportional to the telomere length (Poon *et al*., 1999). The integration region is determined by growing a sphere on top of the found coordinate. After every step of growth (iteration), the sum under this volume (the telomere) is subtracted by the sum immediately surrounding it (background level). When the process of the growth of the sphere does not contribute to an integrated intensity increase, the algorithm stops and the integrated intensity of the telomere with an automatic background correction is obtained.

The telomeric distribution inside the nucleus is described by fitting an ellipsoid to the volume occupied by the telomeres. The distributions were found to be either oblate or spherical. It is therefore convenient to describe the distribution volume as a spheroid (i.e. an ellipsoid having two axes of equal length). As such, it is simpler to describe the spheroid degree of variation from a perfect sphere by the ratio a/c where a and b are the similar semiaxes and c is the third dissimilar axis. Such a description reflects the degree to which the telomere's volume is oblate. Nuclear flattening that may affect a/c ratios was considered in this study. All cells (samples) in an experiment were processed at the same time. If nuclei on one slide consistently showed high a/c ratios, whereas nuclei on parallel processed slides did not, we assumed that the a/c ratio reflected the flatness of telomere distribution of nuclei within that specific sample. All experiments were performed three times.

SKY analysis

SKY was performed by using the ASI (Applied Spectral Imaging, Vista, CA, USA) kit for mouse and Spectra Cube on a Carl Zeiss Axioplan 2 microscope. At least 20 metaphases were examined per time points.

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Extra View

Oncogenic Remodeling of the Three-Dimensional Organization of the Interphase Nucleus

c-Myc Induces Telomeric Aggregates Whose Formation Precedes Chromosomal Rearrangements

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ABSTRACT

The three-dimensional (3D) organization of the normal interphase nucleus permits the regulated completion of transcription and replication and assures proper chromosome organization. Aberrations from the normal 3D structural order of the nucleus are found in tumor cells. When examining the 3D organization of telomeres in nuclei of normal and tumor cells, we found that telomeres of normal nuclei do not overlap, while telomeres of tumor cells form aggregates of various numbers and sizes. To understand how such changes occur and what their implications are we have recently examined the role of the oncoprotein c-Myc in inducing changes in the 3D nuclear organization of telomeres. We found that c-Myc remodels the organization of telomeres and chromosomes in the interphase nucleus. It induces the formation of telomeric aggregates and fusions that are followed by breakage-bridge fusion cycles, and lead to the onset of chromosomal rearrangements that are typical of tumor cells.

The three-dimensional (3D) organization of the nucleus has long been studied. It has become evident that replication and transcription occur at specific nuclear compartments.[1-3] Furthermore, the majority of laboratories have described a probable and nonrandom organization of chromosomes into chromosome territories.[4-9] Chromosomes have frequent neighbors, and the proximity to other chromosomes is tissue-specific.[8,10]

Chromosome territories are maintained during evolution.[11] Data collected over the past decades indicate that gene-dense human chromosomes are found in the center of the nucleus, while gene-poor chromosomes are found towards the periphery of the nucleus.[6,8,12-15]

Taking all the available information together, it thus becomes evident that nuclear organization is highly specific and has functional relevance to the cell assuring proper gene expression, replication and the stability of the genome.

An altered picture is found in the nuclei of tumor cells. For pathologists, the morphology and shape of tumor nuclei have long been crucial hallmarks for diagnostic evaluation compared to normal nuclei.[16-18] Research laboratories have been trying to define what causes this difference and whether it can be used to understand mechanisms of the structural changes that are relevant to the oncogenic process and to design new diagnostic tools. In the end, the best scenario would be the identification of changes that are diagnostically relevant and to define how they occur so that one can design proper means to interfere with these changes therapeutically.

We have recently examined the 3D organization of telomeres in nuclei of normal, immortalized and tumor cells.[19] This study allowed us to conclude that telomeres of nuclei from normal cells do not overlap. Moreover, telomeres are organized in a cell cycle-dependent manner.[19] In G_0/G_1, they are widely distributed throughout the nucleus of primary mouse and human lymphocytes. In S phase, they occupy this same space. In the G_2 phase, however, telomeres change their nuclear positions and form a new structure that we termed the telomeric disk (TD). In a TD, telomeres align in the center of the interphase nucleus. This novel disk structure is different from the metaphase plate. When the TD forms in G_2, the nucleus is not yet in pro-metaphase, and chromosome condensation has yet to begin. We have therefore proposed that the TD may align chromosomes prior to mitosis and may constitute a new checkpoint.[19]

In contrast to this organization in normal cells, tumor cells have distorted TDs. Tumor cell nuclei show telomeric aggregates (TAs) of various sizes and numbers. Thus, the ordered and non-overlapping 3D nuclear space that telomeres normally occupy is compromised.

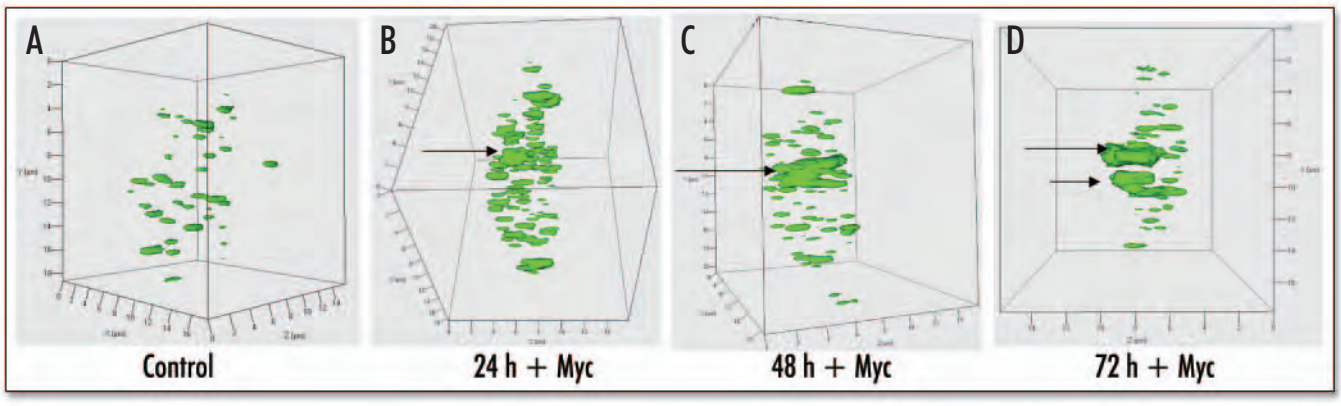

Figure 1. The 3D nuclear organization of telomeres in control (A) and c-Myc activated mouse Pre B lymphocytes (B–D). Telomeres are shown in green. Note the formation of telomeric aggregates as a result of c-Myc deregulation. Arrows point to aggregates. Images were acquired as described.[20]

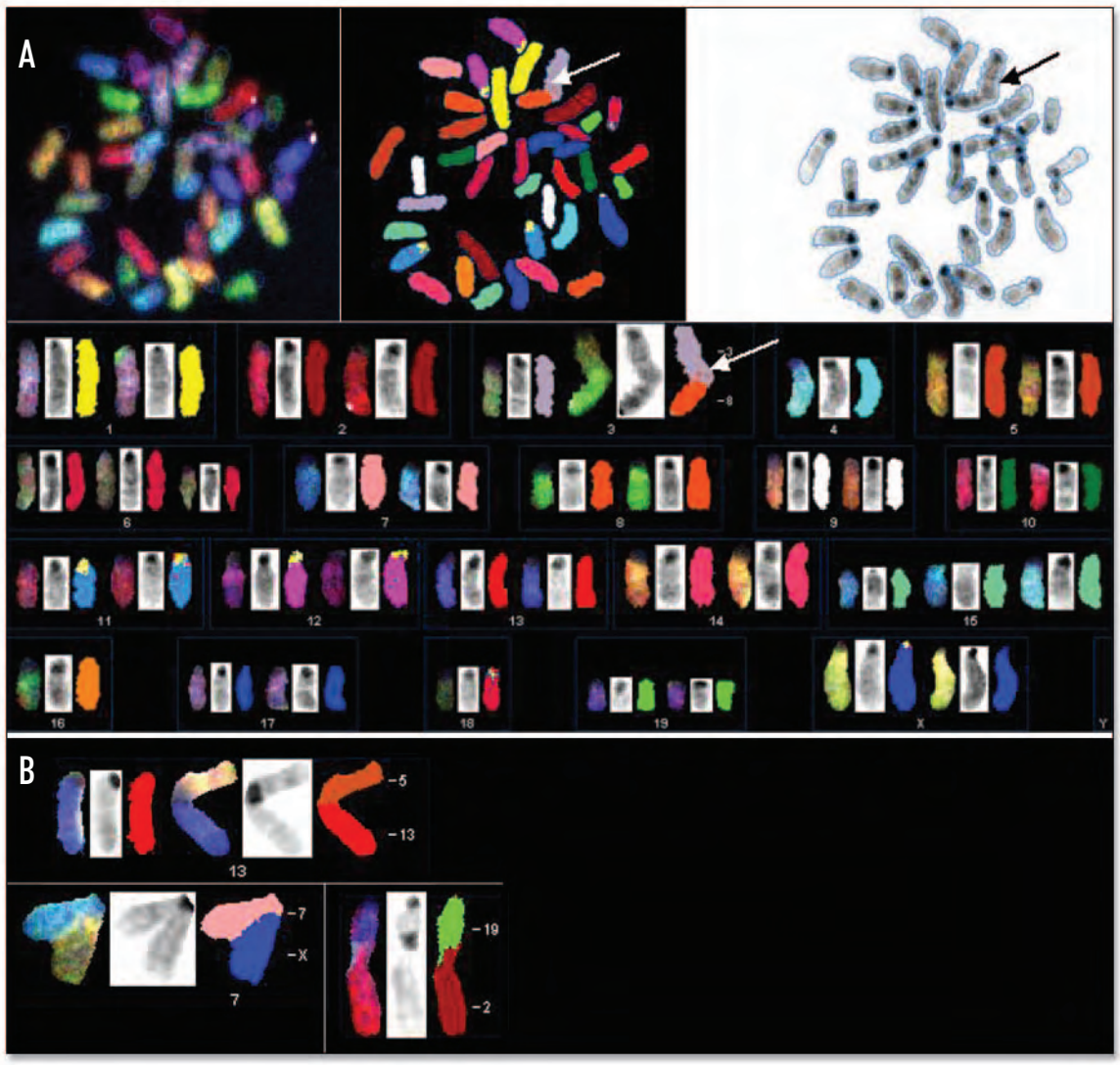

Figure 2. Examples of fused and dicentric chromosomes that form as a result of c-Myc deregulation. (A) Spectral karyotyping (SKY) analysis. c-Myc-dependent formation of a dicentric chromosomes (see arrows). Left panel: raw spectral image of a metaphase, middle panel: spectral image of the same metaphase, right panel: inverted DAPI image of the same metaphase, bottom panel: spectral karyotype of the above metaphase. (B) Additional examples of chromosomal fusions as determined by SKY.

This striking difference between normal and tumor cells prompted us to investigate I) whether the formation of telomeric aggregates was associated with events linked to cellular transformation, and II) whether TAs impacted on the chromosomal organization and genome stability in the 3D interphase nucleus.[20]

We chose to study the role of c-Myc in TA formation since this oncoprotein is associated with at least 70% of human cancers (see ref. 21, and http://www.myc-cancer-gene.org/index.asp). The role of c-Myc in promoting genomic instability has been studied for the past decade, and it is now clear that c-Myc induces a very complex network of genomic instability (reviewed in refs. 22 and 23). For example, c-Myc promotes locus-specific gene amplification[24-28] chromosomal rearrangements,[25,29,30] illegitimate DNA replication,[31] karyotypic instability,[29,32] DNA breakage,[33] and alters DNA repair.[34,35] Since Myc is a multi-functional protein, it also affects transcription (reviewed in refs. 36 and 37), promotes angiogenesis,[38,39] apoptosis[40,41] and alters the immune response of the host so that cells can escape immune surveillance.[42]

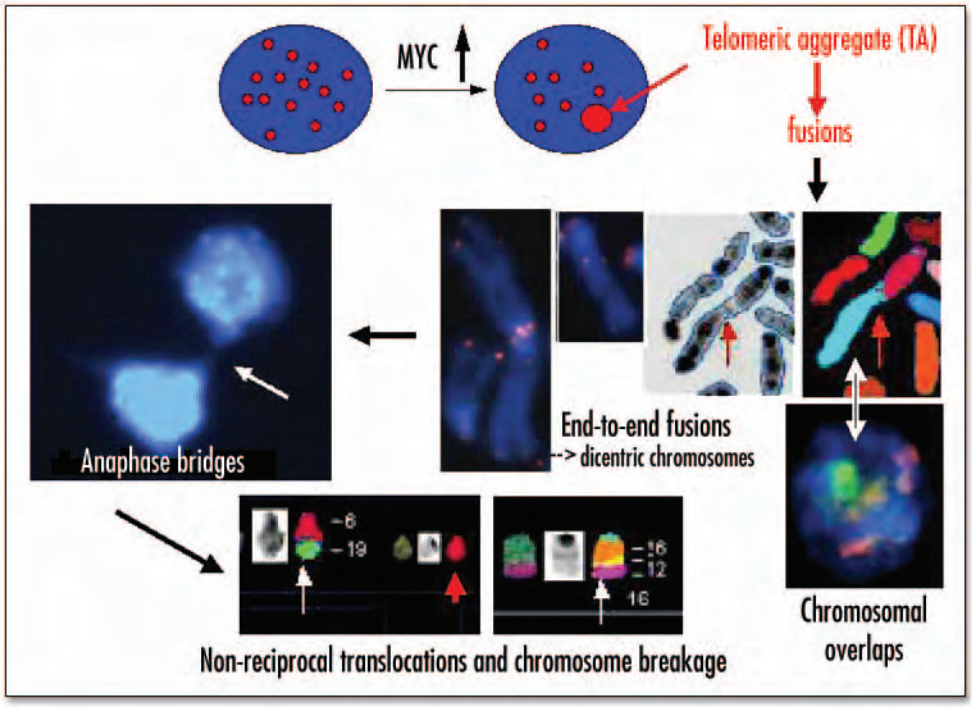

Figure 3. Overview of c-Myc-dependent remodeling of the interphase nucleus. The figure illustrates the formation of telomeric aggregates that represent in part telomeric fusions and move chromosomes into closer vicinity to each other. In anaphase, dicentric chromosomes break generating unbalanced translocations and two telomere-free chromosomal ends. The latter will fuse with new chromosome partners, thus initiating breakage-bridge-fusion (BBF) cycles. c-Myc deregulation induces multiple such BBF cycles.[20]

While deciphering the puzzle of how c-Myc alters genome stability, we have recently shown that c-Myc deregulation can remodel the interphase nucleus by changing the organization of telomeres and chromosomes. These two downstream effects are directly causal to the formation of c-Myc-dependent chromosomal rearrangements.[20] The following findings led to these conclusions.

(1) In cell lines with conditional c-Myc expression, we showed that a single dose 4-hydroxy-tamoxifen (4HT)-activation of MycER^{TM} was sufficient to generate telomeric aggregates in diploid mouse preB cells and in tetraploid Ba/F3 lymphocytes. Both cell types are immortalized but nontumorigenic. In addition, in the absence of c-Myc deregulation, telomeric aggregates in these cells are rare (up to 5% of TAs can be detected). Figure 1 shows an example of telomeric aggregate formation in PreB cells.

(2) Variation of MycER^{TM} activation was also carried out; for example, MycER^{TM} activation was performed for two hours, for 12 hours, every 12 hours, or for the duration of the biological effectiveness of 4HT. All activation schemes lead to the formation of TAs. The formation of TAs was directly proportional to the time of Myc deregulation. c-Myc deregulation induced cycles of TA formation. Fewer cycles (three) were observed after a 2 h pulse of Myc activation than after a 12 h pulse (five cycles).

(3) The formation of TA cycles was most consistent with breakage-bridge-fusion (BBF) cycles as first described by Mueller[43] and McClintock.[44] Chromosomes that fuse at their telomeric ends may form dicentric chromosomes that will break apart during anaphase. The result of this breakage is an unbalanced translocation. Moreover, the ends of the translocation partners are now 'open DNA ends' and represent a double strand break. They are free of telomeres and will fuse with other chromosomes propagating the BBF cycles. Thus, a single deregulation of c-Myc that is as short as 2 hours already leads to the remodeling of the interphase nucleus. Figure 2 shows examples of fused and dicentric chromosomes that were observed as a result of c-Myc deregulation.

(4) As telomeres aggregate, their chromosomes come into closer vicinity. This was measured for the following chromosome pairs: chromosomes 5 and 13, chromosomes 7 and 10, and chromosomes 7 and 17. This close proximity may favor chromosomal rearrangements as we had documented by spectral karyotyping. However, close proximity alone is not sufficient to cause chromosomal rearrangements as chromosomes 11 and 15 that were also found in close proximity and showed mixed color signatures in 3D imaging were only occasionally involved in rearrangements. Figure 3 summarizes the series of events that lead to chromosomal rearrangements.

The previous findings are based on quantitative analyses of the 3D measurements that we have performed. The amount of data and its complexity requires quantitative, standardized and convenient image processing analysis, and it is based on a program and algorithms that we have developed for this purpose. Two main algorithms were developed and used, one for measuring the level of telomeric aggregates and one for measuring chromosome overlap in the nucleus as a function of time.

Telomere measurements were done with TeloView^{TM}.[19,45] By using an adequate threshold, the position of each telomere is found. We then calculate the center of gravity and the integrated intensity of each telomere.[19] The integration region is determined by taking into account the limited optical resolution of the microscope and therefore selecting the correct 3D volume that is occupied by each telomere.

Measurements of chromosomal overlaps were performed after 3D image acquisition and constrained iterative deconvolution.[20] Chromosomes were stained by FISH using two different fluorochromes for each of the two chromosome pairs, e.g., 5 and 13. The nuclear volume was determined based on the DAPI counterstain image and measurements are performed only within its volume. We then determined intensity threshold, and calculated the total volume for each chromosome and for each chromosome type, V_1 and V_2. Each of these values is the sum of volumes of the single chromosomes that belong to the same chromosome type. The total volume that is occupied by both chromosome pairs is then calculated, V_0. The ratio of the overlap with respect to each chromosome type is finally found as V_0/V_1 and V_0/V_2. These overlap-ratios however, seems to follow the same trend for each chromosome pair.[20]

Implications. The above data demonstrate that the 3D nuclear organization is altered as a result of c-Myc deregulation. Since similar telomeric alterations were seen in premalignant and tumor cells, we propose that c-Myc deregulation initiates nuclear remodeling consistent with a tumor phenotype in which, as documented,[20] a novel genomic order is brought about through resulting chromosomal rearrangements. Currently, it is not known whether other oncogenes have similar effects on the nuclear organization. Data by others, however, propose nuclear alterations that are observed in association with the *Ras* oncogene.[46,47]

We propose that the remodeling of the nucleus can be used as a sensitive diagnostic for nuclear aberrations that are associated with diseases like cancer. This approach does not require the presence of metaphases and relies on the 3D organization of the interphase nucleus for its analysis of tumor vs. normal cells. Moreover, nuclear remodeling of telomeres may not only play a role in cancer but also in the generation of mental retardation and malformations in which subtelomeric regions are involved.[48]

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Characterizing the Three-Dimensional Organization of Telomeres

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Background: Quantitative analysis can be used in combination with fluorescence microscopy. Although the human eye is able to obtain good qualitative results, when analyzing the spatial organization of telomeres in interphase nuclei, there is a need for quantitative results based on image analysis.

Methods: We developed a tool for analyzing three-dimensional images of telomeres stained by fluorescence in situ hybridization in interphase nuclei with DNA counterstained with 4′,6-diamidino-2-phenylindole. After deconvolution of the image, we segmented individual telomeres. From the location of the telomeres we derived a distribution parameter ρ_T, which indicated whether the telomeres were in a disk ($\rho_T \gg 1$) or not ($\rho_T \approx 1$). We sorted mouse lymphocyte nuclei and measured ρ_T. We also performed a bromodeoxyuridine synchronous cell sorting experiment on live cells and measured ρ_T at several instances.

Results: Measuring ρ_T for nuclei in G0/G1, S, and G2 produced 1.4 ± 0.1, 1.5 ± 0.2, and 14 ± 2, respectively, showing a significant difference between G2 and G0/G1 or S. For the bromodeoxyuridine synchronous cell sorting experiment, we found a cell cycle dependency of ρ_T and a correlation between ρ_T and an observer.

Conclusions: In this study we present a quantitative method to characterize the organization of telomeres using three-dimensional imaging, image processing, and image analysis. © 2005 International Society for Analytical Cytology

Key terms: telomeres; three-dimensional imaging; image processing; fluorescence microscopy; fluorescence in situ hybridization

Mack Fulwyler was a pioneer in the introduction of modern technology for the analysis of cells and cellular constituents. His work was essential in turning qualitative descriptions in biology into quantitative ones. Further, he understood how it was possible to use these quantitative descriptions to study the dynamics of cellular processes. His interests were not limited to the use of fluorescence in flow cytometry; he realized how fluorescence digital imaging microscopy could provide the tools to answer many questions that were not approachable through fluorescence-activated cell sorting. He also saw how the combination of the two, flow cytometry and image cytometry, could provide an even more powerful mechanism for studying what we have come to know as system biology.

With the advent of sequence-specific DNA probes, the use of fluorescence microscopy in cancer and genetics research has steadily grown. Continuous improvements in fluorescence microscopic methods (hardware and software), specific labeling methods (wetware), and better understanding of the genome function and structure (bioinformatics) currently enable us to detect almost any DNA sequence, gene, or chromosome region with high sensitivity and to address the central question: "What does it mean?"

Because fluorescence methods in most cases are based on digital imaging, quantitative analysis can be used and has become a crucial part of the methodologies. These

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Table 1
*Characteristics of Microscope System**

$FWHM_{lateral}$	200 nm
$FWHM_{axial}$	400 nm
Δx	106 nm
Δy	106 nm
Δz	200 nm
M	63×
NA	1.4
Filters	DAPI, Cy3
Typical image size	200 × 200 × 100 pixels

*FWHM, full width at half maximum; M, magnification; NA, numerical aperture.

methods, therefore, require suitable quantitative image analysis procedures and algorithms. As one of the last links in the chain, the algorithms being used must take into account the entire procedure that is being used, including the optical properties of the microscope and system, nature of the probes, and instrument parameters for the acquisition.

The organization of the interphase nucleus has been studied since the late 19th century (1). It is now well accepted that the position of chromosomes in the nucleus plays an important role in gene regulation (2). Recently, interest has also focused on telomeres whose importance to genomic stability was recognized as early as in the 1930s (3).

We have developed a method of studying the spatial organization of the genome in the three-dimensional (3D) interphase nucleus using flow sorted living cells. We analyze digital images of the 3D organization of the telomeres and how their positions change during the cell cycle. This method enables us to determine for the first time that telomere organization is cell cycle dependent with assembly of telomeres into a telomeric disk in G2 phase. Further, this disk formation is disrupted in tumor cells (4).

In this work we describe in more detail the algorithms that have been developed for the quantitative analysis of the telomeres in interphase nuclei. It can be extended to include centromeres and whole chromosomes.

MATERIALS AND METHODS

Cell Preparation

We studied two different cell nuclei populations based on mouse B lymphocytes. In the first case, immortalized mouse B lymphocyte cells were sorted according to their DNA content for the determination of G0/G1, S, or G2 phase. Cell cycle fractions were quantified through fluorescent-activated cell sorted analysis (4). Flow analyses were performed on an EPICS Altra cytometer operating under Multicycle software (Beckman-Coulter, Paris, France). Approximately 10 to 15 nuclei from each phase were analyzed for this study, representing a total of 35 cell nuclei.

To further study the phase transition timing along the cell cycle, we used the synchronous bromodeoxyuridine (BrdU) sorting method (4). The mouse B lymphocytes were labeled in vivo with BrdU. All BrdU-positive cells (i.e., cells in S phase, replicating their DNA) were live sorted and placed into culture. Populations of nuclei were then harvested at different times (3, 3.5, 4.5, 5.5, 6.5, 7.5, 8, 8.5, and 9.5 h) of which approximately 20 nuclei were analyzed, representing a total of 180 cell nuclei.

For measurement of the telomeric disk, cells were first fixed and then telomere fluorescence in situ hybridization (FISH) was performed as describe previously (5) using a Cy3-labeled peptide nucleic acid (PNA) probe (DAKO, Glostrup, Denmark). 4′6-Diamidino-2-phynylindole (DAPI) was used as a DNA-specific counterstain. Telomere hybridizations were specific and we verified the correct number of telomeric signals observed at the ends of chromosomes prepared from primary cells using two-dimensional (2D) FISH metaphase spreads. The lymphocytes were fixed in such a way that the 3D structure of the nuclei was conserved (4).

3D Image Acquisition

For analysis of the telomere distribution, images were acquired with a Zeiss Axioplan 2 with a cooled AxioCam HR CCD in combination with a PlanApo 63×1.4 oil immersion objective (Zeiss). This gave a pixel (sampling) distance in the lateral plane of $\Delta x = \Delta y = 106$ nm. The axial sampling distance between planes was $\Delta z = 200$ nm. The point spread function (*psf*) of the objective, which determines the optical resolution, gave a full width at half maximum of approximately 200 nm in the lateral direction and 400 nm in the axial direction. Typical image size was 200 × 200 × 100 pixels. Table 1 shows a summary of these values for this imaging system.

Figure 1 illustrates the system resolution. An image of a pair of telomeres relatively far apart and an image of a pair close together are shown. It is clear that the telomeres at a distance of 1200 nm can be easily distinguished and telomeres at a distance of 400 nm are just barely separable.

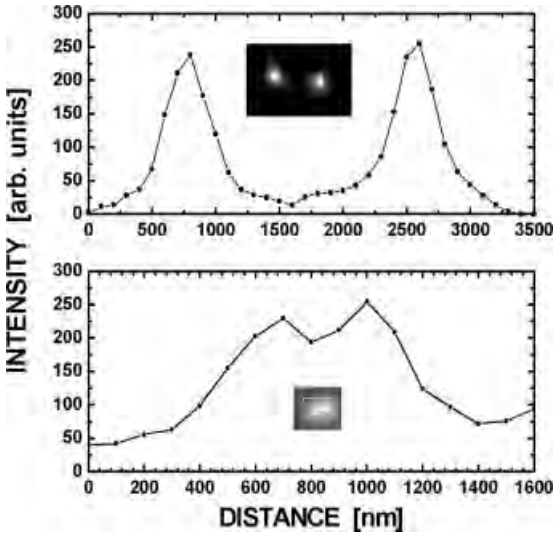

FIG. 1. Demonstration of the spatial resolution of our measurements. Two pairs of telomeres are shown: 1200 nm apart (top), which can be easily separated, and 400 nm apart (bottom). Inserts show the original image and graphs show the line section though the telomeres.

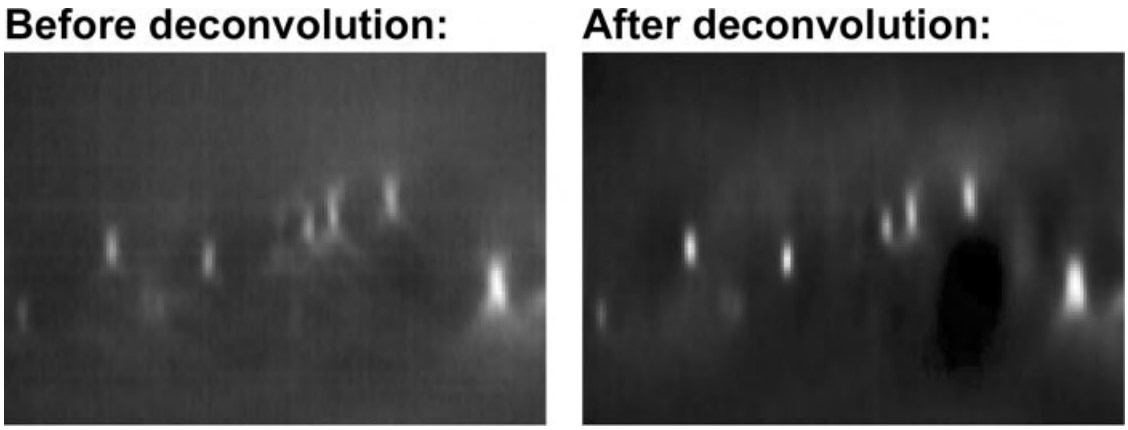

FIG. 2. Demonstration of the effect of deconvolution. The left image is before deconvolution and the right after deconvolution. We clearly see that the left image has more blur and has less contrast than the right image. Both images are shown with a linear contrast stretch.

3D Image Processing

The 3D digital images were processed to improve the resolution by using constrained iterative maximum likelihood deconvolution (6), which is available in AxioVision 3.1 (Zeiss) software. This deconvolution method was chosen for this work because it has been shown to provide the best results (7). In this procedure we seek to find the most likely original image that could have produced the observed data. This devolves into minimizing a measurement between the recorded image and a blurred estimate of the object assuming (a) a certain model for the image noise (Poisson) and (b) separate estimates for the background and the *psf*. Poisson noise is an excellent model for the random variations found in fluorescent images acquired through high-quality CCD cameras. Our estimate for the *psf* was based on a theoretical calculation (8). In this specific case we work with the log-likelihood function $\phi(\mathbf{f})$ which is given by:

$$\phi(\mathbf{f}) = \sum \mathbf{Hf} - \mathbf{g}^T \ln(\mathbf{Hf} + \mathbf{b}) + \gamma|\mathbf{f}|^2 \qquad (1)$$

where $\mathbf{g}$ is the digital fluorescence image that was recorded, $\mathbf{b}$ is an estimate of the digital image background, $\mathbf{H}$ is the *psf* of the imaging system, γ is a scalar "regularization" parameter that we are free to choose, and $\mathbf{f}$ is the original image that we would like to estimate. Equation 1 is a concatenation of equations 4 and 5 in Verveer et al. (7). The iterative algorithm seeks an image $\mathbf{f}$ that minimizes $\phi(\mathbf{f})$ and thereby produces the most likely $\mathbf{f}$ that could have given rise to the measured $\mathbf{g}$.

Further, the deconvolution works with the constraint that the final restored image should consist of only nonnegative numbers because we cannot have a negative number of photons. An example of the result of applying this procedure to telomere images is shown in Figure 2. After restoration, the image is interpolated in the axial (z) direction from Δz to $\Delta z'$ so that the sampling distance in all three directions is the same: $\Delta z' = \Delta x = \Delta y = 106$ nm.

3D Image Segmentation and Analysis

Image segmentation and analysis of deconvolved 3D images of cells with labeled telomeres have been performed with a sequence of procedures that we have bundled together and named TeloView. The procedures themselves are from our image software library DIPImage, which is available as public domain software (http://www.qi.tnw.tudelft.nl/DIPlib/). The version of DIPImage used in this development operates under MatLab (The MathWorks, Natick, MA, USA).

TeloView loads the 3D image and displays a maximum projection along the three main optical axes. Although thresholds and other parameters can be adjusted for display purposes, the analysis is performed on the original 3D data. After segmentation the 2D display indicates the location of the automatically found spots for verification. The user interface is shown in Figure 3.

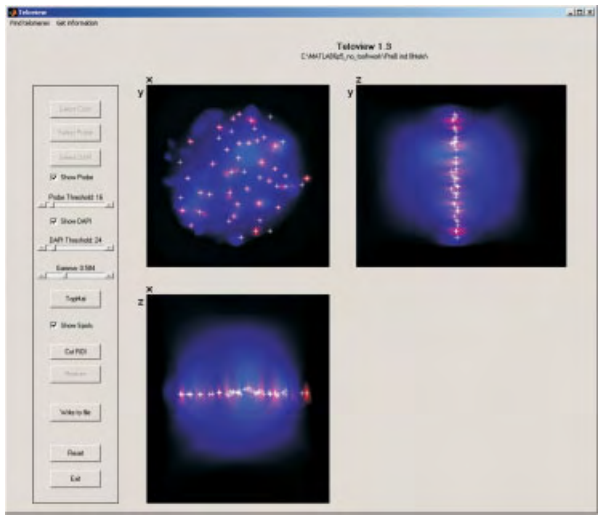

FIG. 3. Screenshot of the interface of TeloView. The screen shows three displays with maximum intensity projections along the three main axes. It also shows crosses at the locations where the software identified a telomere.

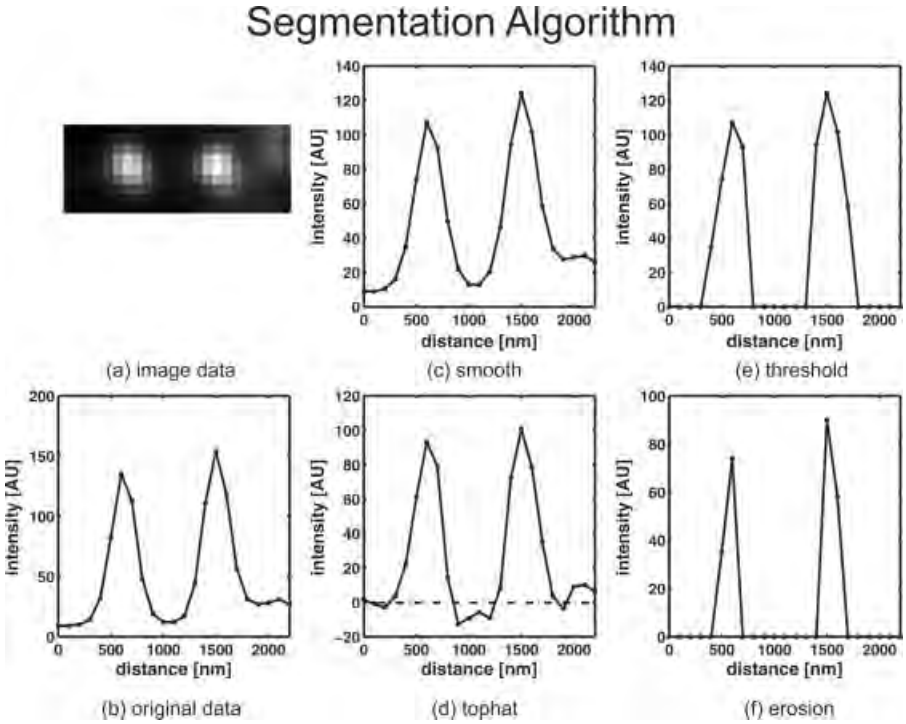

FIG. 4. Working of the algorithm. First we see the raw "image data." A line through the center of this image gives a line section seen in "original data." After we "smooth," we perform a "TopHat" transform. Note that shading is now removed. We "threshold" and end up with two spots. One last "erosion" is performed to make sure that there are no remaining noise spikes.

Segmentation

Before starting the segmentation we pre-process the data by smoothing with a 3D Gaussian kernel. Figure 4 shows how the data are transformed during the different steps of segmentation. For segmentation of the individual telomeres we have chosen an algorithm based on a morphologic TopHat transformation (9,10). The TopHat transform on an image A with structuring element B is defined as follows (11).

To find objects with high intensity ("light" objects):

$$\text{TopHat}(A,B) = A - \max_B(\min_B(A)) \qquad (2)$$

To find objects with low intensity ("dark" objects):

$$\text{TopHat}(A,B) = \min_B(\max_B(A)) - A \qquad (3)$$

The "structuring element," B, can be a quite general 3D gray-value object, but in our case we have chosen for the simple case that B is spherical. B should be larger than the objects that are being sought but smaller than any shading in the background. For a gray-scale image of telomeres, the telomeres would be our objects and any nonspecific binding of Cy3 uniform spread over the nucleus gives us shading. Thus, for our case, this translates to a spherical B with radius 742 nm (7 pixels).

After the TopHat transform, the resulting image is thresholded with a user-chosen value to produce a binary mask. To eliminate noise spikes that may remain, we conclude with an erosion. This algorithm gives satisfying results for small telomeres. Using the binary image mask from the segmentation, the center of gravity of each dot is found. This gives coordinates (x_i, y_i, z_i) for each individual dot, where i is the index number of the dot.

Analysis

Observing the organization of telomeres in many cells, we see that the envelope shape of the telomeres is usually a spheroid, as illustrated in Figure 5. A spheroid is a geo-

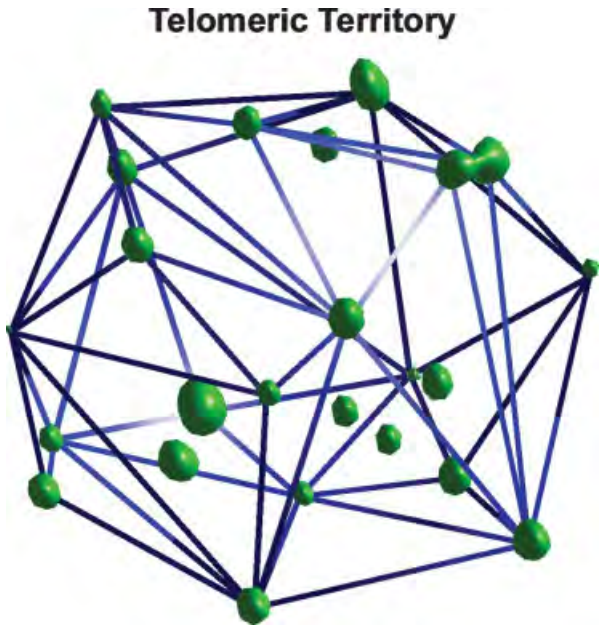

FIG. 5. The telomeric territory can be given by a convex body containing all the telomeres. In most cases this envelope can be approximated by a geometric figure called a spheroid.

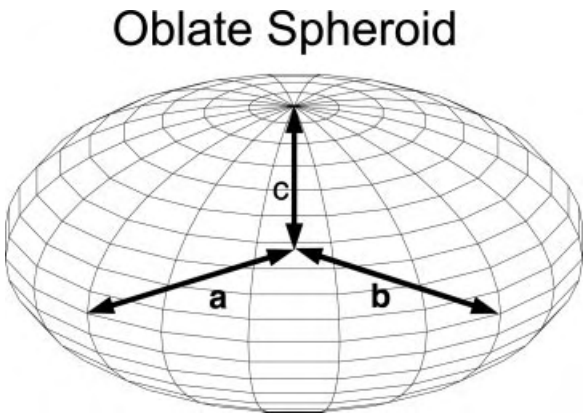

FIG. 6. The telomeric territory is characterized as an oblate spheroid, where two of the main axes, a and b, are of equal length and the third main axis, c, is shorter. The ratio ρ_T = a/c now gives a measure of the flatness of the spheroid.

metric figure, like an ellipsoid, where the two main axes, a and b, are equal, and the third axis c has a different length, c. This is shown in Figure 6. If $a = c$, we have a sphere; if $a < c$, we have a prolate spheroid; and if $a > c$, we have an oblate spheroid. We can therefore define a telomere ratio parameter, ρ_T, which gives us a measurement of the disk-like nature of this organization. If $\rho_T \approx 1$, then the telomeres are distributed in a spherical way within the cell. However, if $\rho_T > 1$, then the telomeric territory is more disk-like. In the following, we describe how we determine ρ_T.

Given the spatial coordinates of every spot $(x_i, y_i, z_i \mid i = 1, 2, \ldots, N)$, we rotate the original spatial coordinates (x, y, z) to a new orthogonal coordinate system such that the distance from the spots to the new axes is minimized. This procedure is known as a principal component analysis (12). To accomplish this, we calculate the singular values (eigenvalues) of the covariance matrix of the data points. The three singular values, $\lambda_1 \geq \lambda_2 \geq \lambda_3$, are real and positive and can be ordered. They are the variances of the distances from the spots to the new principal axes. The standard deviation for each new axis is then given by:

$$\sigma_i = \sqrt{\lambda_i} \tag{4}$$

From these standard deviations, we then define ρ_T as:

$$\rho_T = \frac{\sqrt{\sigma_1 \sigma_2}}{\sigma_3} \tag{5}$$

Given that we work with ordered λs, we have:

$$\rho_T \geq 1 \tag{6}$$

RESULTS

Results of the analysis of the cell-sorted mouse lymphocytes are presented in Table 2. Here we see small values (close to 1) of ρ_T for nuclei in G0/G1 and S phases, which indicate that telomeres are distributed throughout the cell. For the telomeres in G2, however, there is a high value of ρ_T, indicating that the telomeres form a disk. A statistical analysis, using a two-sample Student's t test with unequal variances, indicates a significant difference in ρ_T between G0/G1 and G2 phases ($P < 0.01$) and between S and G2 phases ($P < 0.01$). An example of typical distributions of telomeres in lymphocytes is shown in Figure 7.

Table 2
Results of Phase-Sorted Cells

Phase	ρ_T
G0/G1	1.4 ± 0.1
S	1.5 ± 0.2
G2	14 ± 2

The results of the BrdU synchronization experiment can be seen in Figure 8. In the left graph we see the results of nuclei counted by a human observer. The observer was presented with a 2D computer display of the 3D distribution. A display of the DAPI counterstain indicated the position of the total DNA.

The nuclei are divided into three groups: (a) nuclei with telomeres in a disk, (b) nuclei in mitosis, and (c) other nuclei (interphase nuclei without a telomeric disk). At 3.5 h, 90% of nuclei show a telomeric disk. Based on timing since S phase, most nuclei are believed to be in late G2. After this point, cells continue through the cell cycle, enter into prometaphase and metaphase (i.e., mitosis), and the number of cells in G2, accordingly, decreases. This correlates with the observation that the fraction of cells with a telomeric disk decreases and the number of cells going into mitosis increases.

In the right graph of Figure 8 we show the result of ρ_T calculations on the same population of nuclei. We also

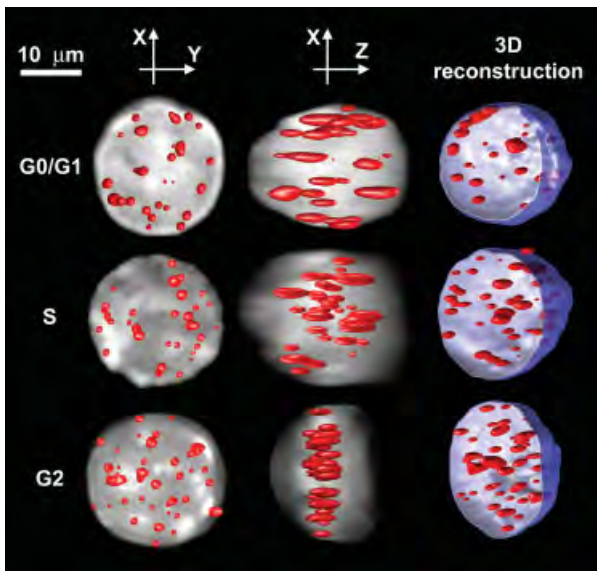

FIG. 7. Three typical distributions of telomeres. A nucleus is seen in G0/G1 phase (top), S phase (middle), and G2 phase (bottom). Every nucleus is shown in top view (xy plane), in side view (xz plane), and as a 3D visualization. We clearly see that the telomeres in G0/G1 and S phases are distributed throughout the nucleus, whereas those in G2 phase line up into a disk.

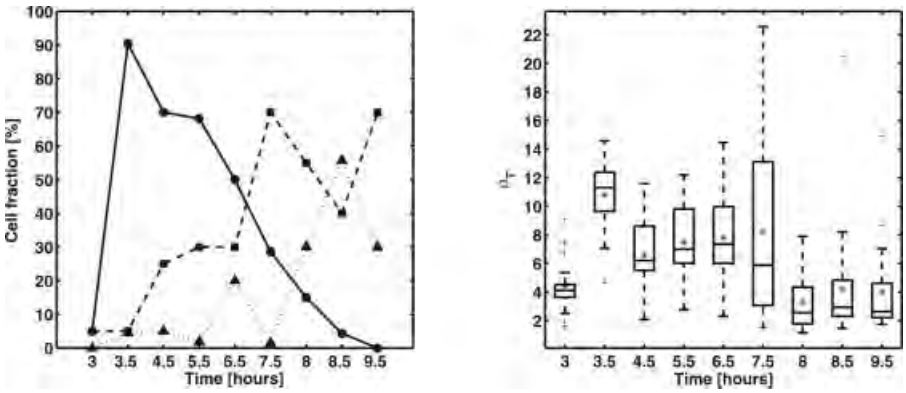

FIG. 8. Results of synchronous BrdU sorting experiment. In the left graph we show the results obtained by a human observer. For each time point approximately 20 nuclei were analyzed and sorted into 3 categories: nuclei with a telomeric disk (black line with circles), nuclei in mitosis (dashed line with squares), and nuclei in interphase without a telomeric disk (dotted line with triangles). In the right graph we show a box plot of ρ_T calculations on the same population. Asterisks indicate the mean ρ_T at every time point and boxes and whiskers represent the 0th, 25th, 50th, 75th, and 100th percentiles of the measurement. Plus signs denote outliers.

observe that the *spread* of ρ_T increases and reaches a maximum at 7.5 h. This coincides with the left graph, where we see an increase in the number of cells without a telomeric disk relative to the number with a telomeric disk. The right graph, however, does not correspond exactly to the left graph calculated by the observer because it calculates the average ρ_T value of the complete population of nuclei at that time point; the nuclei can be in different cell phases. By using a threshold on the ρ_T value, it is possible to imitate the classification of nuclei in a disk. If we choose a threshold of 6.7 between nuclei in a disk ($\rho_T >$ 6.7) and others, we can calculate the fraction of these nuclei and get approximately the same curve as the human observer got. Apparently this is the subjective threshold that was selected when the left graph of Figure 8 was created.

In Figure 8 we notice that the fraction of cells in mitosis at 7.5 h is twice as high as those with a disk. In Figure 9 we see the histogram of the data at 7.5 h, which suggests two populations with twice the amount of low ρ_T cells compared with high ρ_T cells. In the box plot in Figure 8, we also see outliers that are easily explained if the data within the box and whiskers are from the population with low ρ_T and the outliers are from the population with high ρ_T. The outliers in Figure 8 at $t = 8.5$ h, for example, are due to the last few cells from the G2 population that have not yet entered mitosis.

DISCUSSION

For the first time we show that the telomere organization in the nucleus can be characterized and is cell cycle dependent. It is very important to recognize the need for 3D image processing for quantification where objective measurements are preferable above the subjective view of an individual. We have developed an objective means to quantify and analyze the spatial arrangement of telomeres, a task that is, essentially, too difficult for ordinary human vision that can only do qualitative estimates. This is done by calculating a parameter, ρ_T, whose value measures the disk-like compactness of the telomere distribution. We show that ρ_T is significantly higher in cells in G2 than in G0/G1 or S, which suggests that the telomeres form a disk during G2. This is also observed in a synchronous BrdU sorted population, where high ρ_T values are observed after 3.5 h and where the disk phenomenon decreases as cells leave G2. Although a human observer can only threshold the data into fractions called "disk" or "no disk" with a subjective threshold, our method allows this threshold, but we can distill more information out of the data like the distribution of the ρ_T values. It may be valuable in the future to combine these data with the DAPI intensity distribution that may allow distinguishing nuclei in mitosis (based on intensity uniformity) from the other cell cycle phases.

These findings shed new light on the cell cycle mechanism. It remains to be determined whether the telomeric disk is a precursor structure that will later position the chromosomes into the equatorial disk.

Nevertheless, we have already observed that, in cancer cells, the telomeres tend to form aggregates (4), which is another indication to the large role that the organization of the genome in the nucleus has in cancer development.

We are developing methods to quantify the size of a telomere or telomere aggregates, where telomeres are found in close association, as previously shown (4). It is therefore important to quantify such aggregates and test their correlation with cancer progress. Aggregates are usually significantly larger then individual telomeres. As a result, our current algorithm tends to miss these spots because

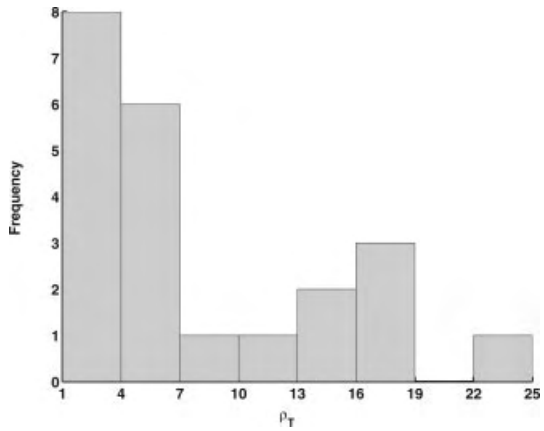

FIG. 9. Histogram of measured ρ_T at 7.5 h after BrdU pulse labeling. The histogram suggests two populations: one with low ρ_T and one with high ρ_T.

the TopHat transform is sensitive to the size of the telomeres. Therefore, we are currently working on an improved segmentation procedure based on a scale-space algorithm (13) that looks most promising. Another suggested path of research is to follow telomeres through the entire cell cycle in living cells.

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c-Myc induces chromosomal rearrangements through telomere and chromosome remodeling in the interphase nucleus

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In previous work, we showed that telomeres of normal cells are organized within the 3D space of the interphase nucleus in a nonoverlapping and cell cycle-dependent manner. This order is distorted in tumor cell nuclei where telomeres are found in close association forming aggregates of various numbers and sizes. Here we show that c-Myc overexpression induces telomeric aggregations in the interphase nucleus. Directly proportional to the duration of c-Myc deregulation, we observe three or five cycles of telomeric aggregate formation in interphase nuclei. These cycles reflect the onset and propagation of breakage-bridge-fusion cycles that are initiated by end-to-end telomeric fusions of chromosomes. Subsequent to initial chromosomal breakages, new fusions follow and the breakage-bridge-fusion cycles continue. During this time, nonreciprocal translocations are generated. c-Myc-dependent remodeling of the organization of telomeres thus precedes the onset of genomic instability and subsequently leads to chromosomal rearrangements. Our findings reveal that c-Myc possesses the ability to structurally modify chromosomes through telomeric fusions, thereby reorganizing the genetic information.

genomic instability | 3D nucleus | breakage-bridge-fusion

Multiple alterations accompany tumor initiation and progression resulting in the modulation of gene expression and in genomic instability. These interconnected changes occur within nuclei that harbor an altered 3D organization (1–3). In agreement with this concept, recent reports suggest tumor-associated changes of chromosomal organization in an altered 3D nucleus (3–8). However, mechanisms leading to structural changes of telomeres and chromosomes remain elusive.

We recently reported that the normal interphase nucleus has a unique 3D telomeric organization that is cell cycle dependent (9, 10). Telomeres are organized in a nonoverlapping manner and align into a central telomeric disk during the late G_2 phase of the cell cycle (9). In contrast, tumor cells display an aberrant organization of telomeres that can be objectively measured in nuclei showing telomeric aggregates of various complexity and sizes (9).

Constitutive expression of c-Myc due to chromosomal translocations, mutation, or amplification contributes to the development and progression of many cancers (11, 12). c-Myc deregulation directly promotes genomic instability (13), causing locus-specific and karyotypic instability (14–18). Additionally, c-Myc induces illegitimate replication initiation (19, 20), DNA breakage (21), alterations of DNA repair (22, 23), and a low level of point mutations (24, 25). Effects of c-Myc on genomic instability are reversible after a transient experimental activation of c-Myc (15). However, c-Myc continues to generate instability after constitutive deregulation (16). *In vivo*, c-Myc deregulation directly initiates and promotes tumorigenesis (26–30). When c-Myc deregulation is abolished, *in vivo* tumorigenesis is reversible, provided that no additional mutations had occurred (29–34).

Prompted by the complexity of downstream genetic alterations that result from c-Myc deregulation, we investigated whether c-Myc affected the 3D organization of the mammalian interphase nucleus and whether this remodeling had an impact on genomic stability. We show that c-Myc deregulation causes remodeling of the 3D nuclear organization of telomeres and chromosomes, thus creating the topological conditions that initiate genomic instability.

MEDICAL SCIENCES

Materials and Methods

Cells and Conditional Myc Activation. Culture conditions have been described for Ba/F3 (35) and PreB (36) cells. The plasmacytoma cell line MOPC460D was a gift of J. Mushinski (National Institutes of Health, Bethesda). Cell viability was determined by hemocytometer counts by using trypan blue. The primary mouse plasmacytoma DCPC21 was isolated from a BALB/c mouse (37). *v-abl/myc*-induced plasmacytomas (38) and primary lymphocytes were collected from BALB/c mice (Central Animal Care protocol 02-039).

To activate MycER (39) in Ba/F3 or PreB cells, 10^5 cells per ml were treated with 100 nM 4-hydroxytamoxifen (4HT). Cells were split 24 h before 4HT treatment. Non-4HT treated control cells were cultivated in ethanol, which is used to dissolve 4HT (25, 26, 39). Two different MycER activation schemes were performed. First, analyses of c-Myc-induced changes in 3D telomere organization were carried out after a single addition of 4HT that was left in the culture medium until its biological effects subsided (40– 42). Nuclei were examined every 24 h over a 10-day period. A second time course was performed every 6 h for 120 h (Fig. 1). To enable a time-dependent analysis of Myc activation, 4HT was given for 2 or 12 h and was removed. Alternatively, 4HT was added every 12 h or was given once but left in the culture. MycER activation was determined by fluorescent immunohistochemistry.

Immunohistochemistry (IHC). Fluorescent IHC of Myc protein was performed as described in ref. 43 by using a polyclonal anti-c-Myc antibody (N262; Santa Cruz Biotechnology) and a goat anti-rabbit IgG FITC antibody, each at a dilution of 1:100. Analysis was

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Abbreviations: 4HT, 4-hydroxytamoxifen; SKY, spectral karyotyping; TA, telomeric aggregate.

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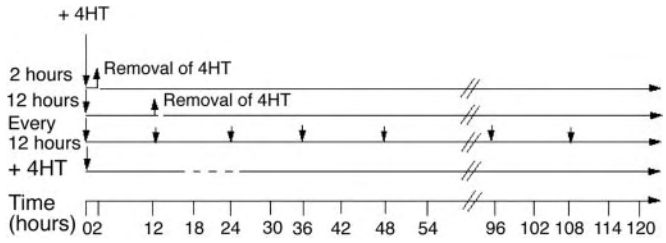

Fig. 1. MycER activation scheme. The effects of 4HT last 15–24 h in cell lines (40–42), as indicated by dashed lines. Cells were harvested every 6 h over a time period of 120 h. Mock-treated control cells were processed in parallel.

performed by using a Zeiss Axiophot 2 microscope. Images were acquired with a Cooke CCD SensiCam Camera.

Cell Death. Apoptotic bodies for control and MycER-activated cells were assessed by two independent observers who scored 300 DAPI-stained nuclei per time point in the presence or absence of MycER activation.

Telomere FISH. Ba/F3, PreB, and plasmacytoma cells were collected (200 × *g* for 10 min) and resuspended in PBS containing 3.7% formaldehyde (Fluka) and incubated for 20 min. Thereafter, the telomere FISH protocol was performed (9, 44) by using Cy3- or FITC-labeled PNA probes (DAKO). Three independent experiments were performed. At least 30 nuclei and 20 metaphases were examined per time point. Imaging of metaphases after telomere FISH was performed by using Zeiss Axioplan 2 with a cooled AxioCam HR B&W, DAPI, Cy3, or FITC filters in combination with Planapo 63x/1.4 oil objective lens. Images were acquired by using AXIOVISION 3.1 (Zeiss) in multichannel mode. Because of the presence of multiple variables, the general linear modeling procedure was used. To test average aggregates among different groups, a two-way ANOVA test was performed for normality and robustness of the data. For details of all tests preformed, see *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

3D Image Acquisition. At least 30 nuclei were analyzed for each time point. AXIOVISION 3.1 with deconvolution module and rendering module were used. For every fluorochrome, the 3D image consists of a stack of 100 images with a sampling distance of 200 nm along the *z* and 107 nm in the *xy* direction. The constrained iterative algorithm option was used (45).

3D Image Analysis for Telomeres. Telomere measurements were done with TELOVIEW (9, 46). By choosing a simple threshold for the telomeres, a binary image is found. Based on that, the center of gravity of intensities is calculated for every object resulting in a set of coordinates (*x*, *y*, *z*) denoted by crosses on the screen. The integrated intensity of each telomere is calculated because it is proportional to the telomere length (47). The integration region is determined by growing a sphere on top of the found coordinate. After every step of growth (iteration), the sum under this volume (the telomere) is subtracted by the sum just surrounding it (background level). When the process of the growth of the sphere does not contribute to an integrated intensity increase, the algorithm stops and the integrated intensity of the telomere with an automatic background correction is obtained.

Chromosome Painting and Measurements of Chromosomal Overlap(s) in Interphase Nuclei. Chromosome painting was carried out as described in ref. 48 by using paints for mouse chromosomes 5 (Cy3), 13 (FITC), 7 (Cy3), 10 (FITC), and 17 (FITC) from Applied Spectral Imaging (Vista, CA). 3D image acquisition of painted nuclei was performed as described above. Measurements of chromosomal overlaps were performed after 3D image acquisition and constrained iterative deconvolution as follows: (*i*) based on the DAPI counterstain image, we determined the 3D boundary of the nuclear volume. Data outside that volume were ignored. (*ii*) For each one of the chromosomes, we determined an intensity threshold and referred only to voxels that were above the threshold that belonged to the specific chromosomes. The total volume occupied by each one of the chromosome pairs is measured (V_1 and V_2). (*iii*) The volume occupied by both chromosome pairs is measured, V_o. By dividing this value by V_1 and by V_2, the level of overlap relative to the total volume of each chromosome pair was measured, V_0/V_1, V_0/V_2 (for details, see Fig. 8 which is published as supporting information on the PNAS web site).

Spectral Karyotyping (SKY). Mouse SKY was performed by using a SKY system (Applied Spectral Imaging) (37). Twenty metaphases were examined per time point. Significant values for chromosomal rearrangements were determined after MycER activation. Mean total chromosomes and numbers of each chromosome observed for control and Myc-activated cells were compared over time by two-way ANOVA. In addition, statistical analyses were performed for the occurrence of translocations, breakages, and fusions over the experimental period of 120 h. *P* values of <0.05 were considered significant. Only the frequency procedure was used, followed by Fisher's exact test. The *P* value of the overall study was <0.0001.

Supporting Information. For additional information, see Figs. 9–12, Movies 1–3, and Tables 2–4, which are published as supporting information on the PNAS web site.

Results

The 3D Organization of Telomeres Before c-Myc Activation. We examined whether c-Myc deregulation affected the 3D organization of telomeres in the interphase nucleus. To this end, we analyzed the effect of conditional c-Myc expression in two independent immortalized mouse B lymphocyte lines, Ba/F3 (35) and PreB (36), stably transfected with MycER (39). For both cell lines, we first evaluated the 3D organization of telomeres in nuclei of non-MycER-activated cells by using primary BALB/c B lymphocytes as a control. Consistent with our previous studies (9), telomeres of normal primary BALB/c B nuclei showed nonoverlapping telomere posi-

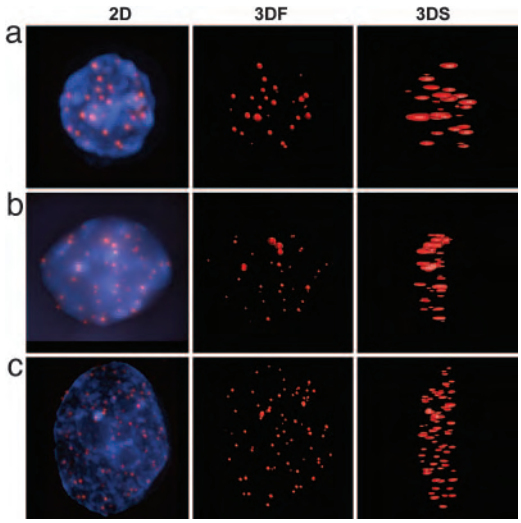

Fig. 2. Telomeric organization in interphase nuclei of primary and immortalized B lymphocytes without overlap in telomere positions. (*a*) Primary B cell nucleus. (*b*) Nucleus of near diploid PreB cell. (*c*) Nucleus of tetraploid Ba/F3 cell. Telomeres are shown in red; nuclei in blue. 3DF, 3D front view; 3DS, 3D side view.

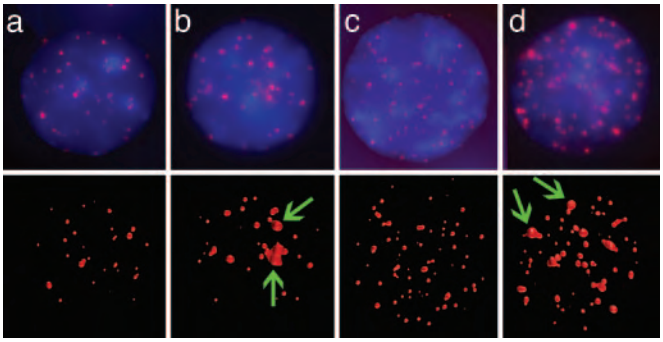

Fig. 3. c-Myc deregulation induces TAs in interphase nuclei of PreB and Ba/F3 cells shown at 72 h after 4HT-treatment. (*a*) Mock-treated PreB cells show nonoverlapping telomeres (red). (*b*) MycER-activated PreB cells with TAs (green arrow). (*c*) Mock-treated Ba/F3 cells show nonoverlapping telomeres. (*d*) MycER-activated Ba/F3 cells show the formation of TAs (green arrow).

tions as determined by 3D imaging (Fig. 2*a*). Without MycER activation, both PreB and Ba/F3 interphase nuclei also displayed nonoverlapping telomere positions (Fig. 2 *b* and *c*, respectively). Therefore, the above cell lines were appropriate to study the effects of conditional c-Myc activation on the 3D telomeric organization.

c-Myc-Dependent Disruption of the 3D Telomeric Organization: Formation of Telomeric Aggregates (TAs) in Interphase Nuclei. We next analyzed the effect of conditional c-Myc expression on the 3D organization of telomeres. After a transient MycER activation with 4HT, nuclear c-Myc signals were observed in both PreB and Ba/F3 cells (Fig. 9 *b* and *d*). In non-4HT treated control cells, MycER was found in the cytoplasm (Fig. 9 *a* and *c*; see also ref. 39).

To determine whether c-Myc deregulation affected the 3D organization of telomeres, we performed time course experiments. In the first set of experiments, c-Myc deregulation and 3D telomeric organization were investigated in both PreB and Ba/F3 cells after a single 4HT treatment. Nuclei were analyzed after c-Myc deregulation at 0, 24, 48, 72, and 96 h and at 10 days and compared with nuclei from mock-treated control cells. In both cell lines, analyses of the 3D nuclear organization of telomeres revealed that c-Myc deregulation induced the formation of TAs. TAs are group(s) of telomeres that are found in clusters and, thus, in close association in the interphase nucleus. This 3D telomeric organization is distinct from the normal 3D organization of non-MycER-activated PreB, Ba/F3 cells and primary mouse lymphocytes (Fig. 2). Fig. 3 illustrates the presence of TAs in interphase nuclei of MycER-activated PreB and Ba/F3 cells (Fig. 3 *b* and *d*, respectively). Although such TAs had been observed in tumor cell nuclei previously (9), their presence in conditional c-Myc expressing cells is a previously uncharacterized finding.

c-Myc Induces Cycles of TAs in Interphase Nuclei. In subsequent experiments, we investigated the time relationship between c-Myc deregulation and the formation of TAs more closely. To this end, cells were harvested every 6 h over a time period of 120 h. We also varied the duration of conditional c-Myc expression (Fig. 1), confirming nuclear c-Myc staining as above (Fig. 9 and 11). Next, the 3D organization of telomeres was determined (Fig. 4). At this point, we focused on near diploid PreB cells only (49). Our positive controls were cells constitutively overexpressing c-Myc [mouse plasmacytomas (27) and a plasmacytoma line (Fig. 4*Ae*)]. Negative controls were mock-treated PreB cells (Fig. 4*Aa*).

This time course confirmed that c-Myc deregulation induced TAs. Representative images show that TAs varied in size and numbers per MycER-activated PreB cell nucleus (Fig. 4*A b–d*, red arrows). High induction levels of TAs were observed at 30, 48, 72, and 96 h declining after 96 h (Fig. 4*B*, arrows). The highest levels

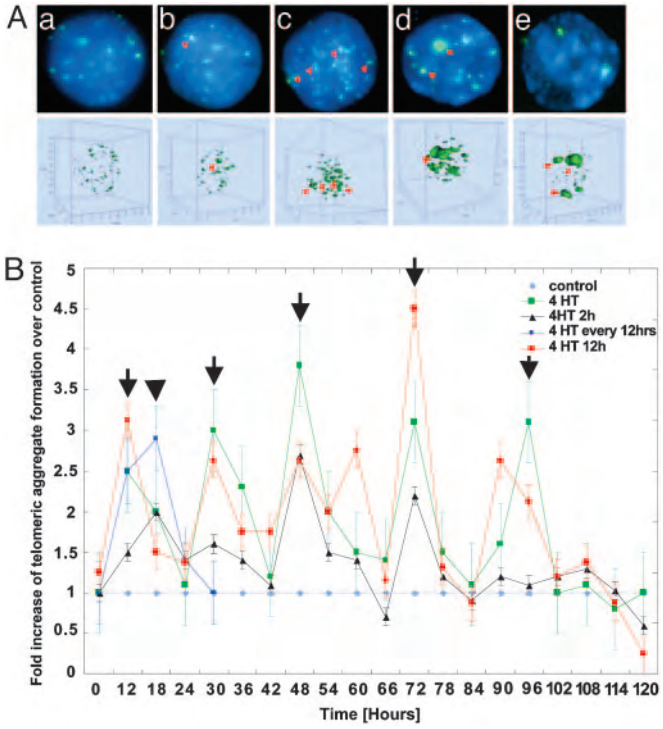

Fig. 4. c-Myc-induced telomeric aggregates appear in cycles. (*A*) Conditional c-Myc deregulation causes TA formation. (*Aa*) Negative control: non-Myc-deregulated PreB nucleus with nonoverlapping 3D telomeric nuclear positions. (*bb–bd*) TAs of various sizes and numbers are present after conditional c-Myc expression at any given time point of TA formation. Telomeres are shown in green; TAs by red arrows. (*Ae*) Positive control: plasmacytoma cell line, MOPC460D, with constitutive c-Myc deregulation due to T12;15, shows TAs. Similar results were obtained with primary plasmacytoma cells (data not shown). (*B*) c-Myc induces cycles of TAs. Fold increase in TAs over control levels during a period of 120 h. During this period, c-Myc had been up-regulated for different lengths of time (see Fig. 1). Black, 4HT given for 2 h and removed; red, 4HT administered for 12 h and removed; green, 4HT added once and not removed; blue, 4HT added at 0, 12, and 24 h; gray, control cells. The highest levels of TA formation and a single TA peak observed after consecutive activations of MycER are shown by arrows and an arrowhead, respectively. Error bars represent a 95% confidence interval of binomial distributions.

of TA formation will hereafter be referred to as peaks of TAs. The 6-h time course performed over 120 h indicated that TAs formed in a c-Myc-dependent manner and showed a cyclic appearance (Fig. 4*B*). The number of TA cycles was directly linked to the duration of c-Myc deregulation. For example, 2 h of Myc activation induced three such cycles, whereas 12 h led to five cycles (Fig. 4*B*, black and red lines, respectively). 4HT, left in the culture medium until its biological effects on our cells subsided (Fig. 1), also induced five TA cycles (Fig. 4*B*, green line). In this context, repeated consecutive activations of MycER given every 12 h caused TAs in 96% of all nuclei. These cells died after 30 h (Fig. 4*B*, blue line) because of repeated cycles of c-Myc deregulation and not due to toxicity exerted by 4HT (50). Thus, only a single TA cycle is observed in this experimental setting (Fig. 4*B*, arrowhead). The increase in TAs and 3D volumes was significant (Table 3).

The c-Myc-Induced TA Cycles Represent Breakage-Bridge-Fusion (BBF) Cycles and Chromosomal Rearrangements. The cycles of c-Myc induced TAs in PreB nuclei showed similar periodicity for all c-Myc activation periods (Fig. 4*B*). We reasoned that these cycles might reflect both ongoing associations and dissociations of telomeres or BBF cycles. The BBF cycle could be induced by the breakage of dicentric chromosomes during anaphase-inducing apoptosis of cells

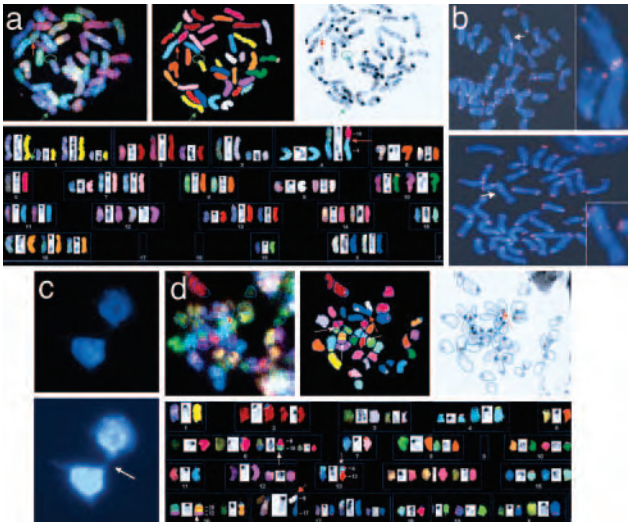

Fig. 5. Molecular cytogenetic evidence of BBF cycles in MycER-activated PreB cells. (*a*) SKY analysis reveals telomeric fusions and chromosome breakage. (*a Upper*) Metaphase, raw image (*Left*); metaphase, classified image (*Center*); and metaphase, inverted DAPI image (*Right*). (*a Lower*) Spectral karyotype. End-to-end fusion of chromosomes 18 and 4 (red arrow) and fusion of chromosome 1 with a broken piece of chromosome 1 (green arrow) are shown. One broken chromosome 1 is circled. Note additional broken chromosomes 1, 2, 3, and 7. (*b*) End-to-end fusions of chromosomes revealed by telomere FISH. (*Upper*) Centromeric fusion (see arrow and insert). (*Lower*) Telomeric fusion (see arrow and insert). (*c*) Anaphase bridges. (*Upper*) Short exposure of DAPI stained nucleus (100 msec). (*Lower*) Longer exposure (500 msec) of same image makes anaphase bridge visible (white arrow) but overexposes nuclei. (*d*) SKY illustrating chromosomal fusions (red arrow) and nonreciprocal translocations (white arrows). Broken chromosomes are also present (chromosomes 4, 6, 12, and 17).

having multiple or large TAs per nucleus. To address these possibilities, we first examined metaphase chromosomes at different times: prior, during, and after the peaks of TA formation for a 120-h period. We used both whole-genome analysis by mouse SKY and telomeric FISH of metaphase chromosomes. A significant level of dicentric chromosomes was noted (Fig. 5). Control cells had normal karyotypes (Fig. 12). In MycER-activated PreB cells, however, fusions had occurred. We show as example fusions at the telomeric ends of chromosomes 18 and 4 (Fig. 5*a*), red and green arrows) and between two chromosomes 1 (Fig. 5*a*, green arrow). Chromosome 1 was probably broken in the previous anaphase (Fig. 5*a*, green circle). An additional terminally deleted chromosome 1 is in the center of the same metaphase plate, and chromosomes 2, 3, and 7 reveal terminal deletions (Fig. 5*a*). Telomeric fusions involving both ends of chromosomes as well as sister chromatids were confirmed by telomeric FISH (Fig. 5*b*). Anaphase bridges and ring chromosomes were present (Fig. 5*c*) and data not shown).

Table 1. Apoptosis levels in non-MycER and MycER-activated PreB cells

	% apoptosis		
Time, h	Controls	MycER-activated PreB cells	Fold increase
0	3.0	3.0	1.0
12	2.0	4.0	2.0
24	6.0	12.0	2.0
30	4.0	10.0	2.5
42	2.0	8.0	4.0
48	5.0	10.0	2.0
66	4.0	11.0	2.75
72	3.0	8.0	2.7
84	3.0	5.0	1.7
96	3.0	3.0	1.0
102	2.0	3.0	1.5

The nature of c-Myc-induced 3D structural changes in interphase nuclei of conditionally Myc expressing cells was as follows: at peaks of TA formation and thereafter, a significant increase in end-to-end chromosomal fusions over control levels was observed. This result was followed by a significant increase in broken chromosomes and nonreciprocal translocations (Figs. 5*d* and 6 and Table 2). In conclusion, TA cycles unveil BBF cycles, namely the fusions of two chromosomes, consequently, the formation of dicentrics and their subsequent breakage in anaphase (Fig. 5). The cycles are induced by conditional Myc deregulation and lead to the onset of genomic instability, demonstrated by the chromosomal rearrangements resulting from these BBF cycles (Figs. 5 and 6 and Table 2).

Next, we investigated whether cells with TAs died during the course of the experiments. If this possibility was the case, we would expect a correlation of cell death in Myc-activated cells at the peak of TA formation or shortly thereafter. The level of apoptosis was ≈2-fold higher in Myc-activated cells than in control cells (Table 1). There was no preference in apoptotic cell death for any specific time point during the 120 h. We concluded that BBF cycles, not apoptosis, contributed to the cycles of TA formation.

3D Organization of Chromosomes in c-Myc Activated Interphase Nuclei. TAs and the initiation of BBF cycles with subsequent chromosomal rearrangements prompted us to investigate whether chromosomes were affected in their 3D nuclear positions during MycER activation. To this end, we examined the overlap of specific chromosomes over the 120-h period. SKY of MycER-activated PreB cells suggested chromosomal rearrangements involving chromosomes 7, 13, and 17. Additional rearrangements were found but

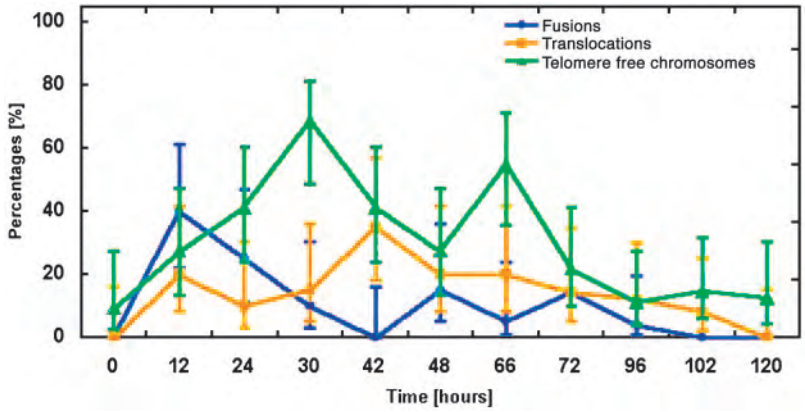

Fig. 6. Chromosomal aberrations in MycER-activated PreB cells over a period of 120 h after a single administration of 4HT. End-to-end fusions (blue) increase to 40% in the first 12 h. Over time, the percentage of fusions decreases. Translocations (orange) appear at 12 h and reach a maximum of 35% at 42 h. Telomere-free chromosomal end(s) (green) increase over time peaking at 30 h with 75% of metaphases having at least one telomere-free chromosomal end. Subsequently, the percentage of telomere-free chromosomal end(s) decreases. Q-FISH experiments confirmed healing of telomeric ends at later time points. The error bars show the 95% confidence interval for binomial distributions (51). Because of a confidence interval, the error bars are larger than expected when a standard error would have been used, which was not applicable in this situation. For details on each time point and aberration, see Table 2.

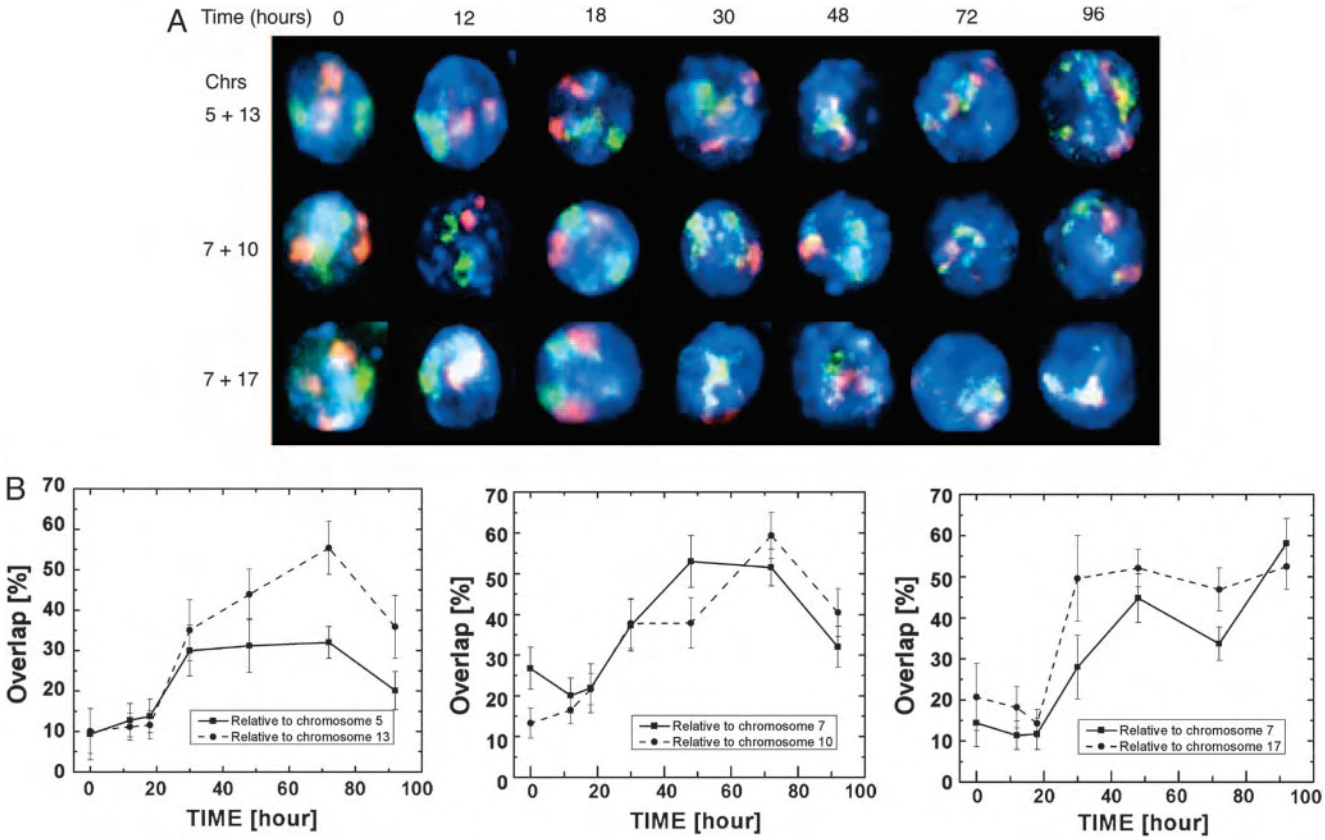

Fig. 7. Chromosome positions in Myc-activated nuclei. (*A*) Representative nuclei painted with chromosome paints over a period of 96 h after 4HT (Fig. 1). (*Top*) Chromosomes 5 and 13. (*Middle*) Chromosomes 7 and 10. (*Bottom*) Chromosomes 7 and 17. (*B*) Measurements of chromosomal overlaps in nuclei of c-Myc deregulated cells for chromosomes 5 and 13 (*Left*), 7 and 10 (*Center*), and 7 and 17 (*Right*) over a 96-h period.

did not reach significant levels (data not shown). We examined three combinations of chromosomes over a 96-h period. This period covered all peaks of TA formation (Fig. 4*B*). As shown in Fig. 7, we observed a change in overlaps between chromosomes 5 (red) and 13 (green) over the time course (Figs. 7 *A* and *B*). Both chromosomes were found in closer vicinity as the cells entered into the first TA cycle. Chromosomes 10 (green) and 7 (red) also showed increases in the percentage of overlap (Fig. 7 *A* and *B*), as did chromosomes 7 (red) and 17 (green) (Fig. 7 *A* and *B*). Representative 3D movies are shown in Movies 1–3.

Discussion

c-Myc Induces Telomeric Aggregates, Fusions, and BBF Cycles. Previous studies have shown that c-Myc triggers a complex network of genomic instability at the level of single genes (14, 15, 19) and whole chromosomes (16–18) (for review, see ref. 13). In addition, c-Myc induces illegitimate replication initiation (19, 20), chromosomal rearrangements (18), DNA breakage, alterations of DNA repair (21–23), and a low level of point mutations (24, 25). A previously uncharacterized mechanism underlying c-Myc-dependent genomic instability at the chromosomal level directly affects the integrity of the telomeres and was revealed in this study.

The clear periodicity of the TA cycles that was found with four different Myc-activating treatments suggested a biological relevant Myc-dependent process. Theoretically, cycles of Myc-induced TAs could reflect (*i*) nuclear remodeling with the transient association and subsequent dissociation of telomeres; (*ii*) end-to-end chromosomal fusions that initiate BBF cycles (52, 53); (*iii*) c-Myc induced cell death; and (*iv*) a combination of all of the above. Our data are consistent with BBF cycles and exclude apoptosis as a direct contributor to the TA cycles. Apoptosis occurred at equal levels throughout the study and consistently reached about twice the levels seen in the control cells. The loss of cells was compensated by a 2-fold increase in proliferation in MycER-activated PreB (19). These data also indicate that there is genetic separation of genomic instability and apoptosis as reported in ref. 54. Whether telomere associations and dissociations (55) contributed to the TA cycles is presently unknown.

Direct evidence of BBF cycles in the periodicity of TAs came from a detailed analysis of chromosomal fusions, breakage, and rearrangements observed over the time course of five TA cycles. We demonstrated the occurrence of end-to-end fusions that generated dicentric chromosomes and breaks during anaphase, leaving one chromosome or chromatid with a piece from another chromosome or chromatid. The resulting telomere-free ends continue to undergo fusions with other chromosomes, a cycle of events termed BBF cycle (52, 53). Experimental data support these events from fusions to breakages and nonreciprocal translocations. The periodicity of the TA cycles is consistent with a ≈12 h population doubling time of the PreB cells (19). Each peak of TAs is consistent with the repeated formation of TAs. Time points after the peak are in agreement with the breakage of dicentric chromosomes. Telomere-free ends initiate new BBF cycle(s) until no more telomere-free chromosomal end(s) persist.

From Telomeres to Chromosomal Rearrangements: A New Pathway of c-Myc-Dependent Genomic Instability. Muller (52) and McClintock (53) first described BBF cycles, a mechanism of chromosomal end-to-end fusion that contributes to the onset of genomic instability. BBF cycles contribute to deletions, gene amplification, nonreciprocal translocation, and overall genetic changes that are associated with tumorigenesis (56–63).

Our study showed that c-Myc is one key factor that initiates genomic instability through BBF cycles. Such BBF cycles in telomerase-positive immortalized mouse PreB cells (unpublished data) with long telomeres are distinct from BBF cycles reported for critically short telomeres (61, 64). Some TAs (but not necessarily all) represent fusions, as evident by the analysis of metaphase chromosomes. TAs and end-to-end fusions depended on time and levels of c-Myc activation. Analysis of frequencies of both events showed that they are closely linked. As the fusions initiate BBF cycles, the frequencies of breakage and nonreciprocal translocations increase over time.

A previously uncharacterized pathway of c-Myc-dependent genomic instability thus starts at the telomeric ends of the chromosomes. Both TAs and BBF cycles are the manifestation of deregulated Myc expression, leading to chromosomal rearrangements and subsequently to genomic instability.

Local chromosome movement increases chromosomal overlap in the nucleus. This temporal change in local positioning may permit the direct contact of chromosomal ends and facilitate recombinations and/or fusions. Such movements were observed after c-Myc deregulation and suggested an impact of the oncoprotein on local nuclear positioning of chromosomes. Chromosome movements were previously studied and found by others as well (65–69).

Several regulatory pathways involving oncogene deregulation may affect the 3D nuclear organization. Oncoproteins, including c-Myc, can alter the 3D nuclear organization and the organization of chromatin (70–72). They also affect the nuclear matrix. High mobility group protein I(Y) (HMGI(Y)) is a c-Myc-dependent nuclear matrix protein (73) with increased expression during neoplasia (2). The analysis of *myc*-binding sites in the human genome suggests that c-Myc binds to genes encoding nucleoskeletal components (74). Furthermore, constitutive c-Myc expression was shown to be associated with down-regulation of the telomere repeat binding protein TRF2 (10), a protein required for telomere capping and genome stability (75). Myc is also involved in the regulation of DNA repair (22, 23) and has been shown to induce DNA breakage (21). Thus taken together, many different c-Myc-dependent mechanisms could potentially affect the nuclear organization and, as shown here, converge at the telomeres.

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Research article

The three-dimensional organization of telomeres in the nucleus of mammalian cells

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Abstract

Background: The observation of multiple genetic markers *in situ* by optical microscopy and their relevance to the study of three-dimensional (3D) chromosomal organization in the nucleus have been greatly developed in the last decade. These methods are important in cancer research because cancer is characterized by multiple alterations that affect the modulation of gene expression and the stability of the genome. It is, therefore, essential to analyze the 3D genome organization of the interphase nucleus in both normal and cancer cells.

Results: We describe a novel approach to study the distribution of all telomeres inside the nucleus of mammalian cells throughout the cell cycle. It is based on 3D telomere fluorescence *in situ* hybridization followed by quantitative analysis that determines the telomeres' distribution in the nucleus throughout the cell cycle. This method enables us to determine, for the first time, that telomere organization is cell-cycle dependent, with assembly of telomeres into a telomeric disk in the G2 phase. In tumor cells, the 3D telomere organization is distorted and aggregates are formed.

Conclusions: The results emphasize a non-random and dynamic 3D nuclear telomeric organization and its importance to genomic stability. Based on our findings, it appears possible to examine telomeric aggregates suggestive of genomic instability in individual interphase nuclei and tissues without the need to examine metaphases. Such new avenues of monitoring genomic instability could potentially impact on cancer biology, genetics, diagnostic innovations and surveillance of treatment response in medicine.

Background

Cancer is characterized by multiple alterations that affect the modulation of gene expression and the stability of the genome. These interconnected changes occur within the nuclei of cells that alter their three dimensional (3D) organization during tumor initiation and progression [1,2]. It seems reasonable to assume that the highly organized mammalian interphase nucleus is the structure that ascertains genomic stability. In line with these concepts, oncogenic activation remodels this nuclear order and sets the stage for genomic instability as we have recently measured for conditional c-Myc deregulation. The deregulated expression of c-Myc alters the 3D nuclear space of chromosomes and telomeres, and makes genomic rearrangements topologically feasible (Chuang *et al.*, in preparation).

Defining the structural organization of the interphase nucleus is, therefore, essential to our understanding of the 3D genome organization in the interphase nucleus. Such a study can be performed by fluorescence *in situ* hybridization (FISH). Two of the most attractive features of FISH measurements of the 3D nucleus organization are the ability to simultaneously visualize multiple targets and the structural organization of nucleus and cells, something that cannot be achieved by array-based methods.

The organization of the interphase nucleus has been studied since the late nineteenth century [3]. It is now well accepted that the position of chromosomes in the nucleus plays an important role in gene regulation [4]. Nevertheless, some controversy exists. Most laboratories have observed a non-random organization of chromosome territories [2,5,6] that has been conserved during evolution [7]. This has been further supported by studies that demonstrate an architectural stability of the chromosomal positions in the nucleus [8,9]. There are, however, different observations on chromosomal positions [10-15] as well as on positional changes of chromosomes during the cell cycle [16,17].

Recently, interest has also focused on telomeres, whose importance to genomic stability was recognized as early as the 1930s [18]. Capping the chromosomes, telomeres are responsible for chromosomal integrity [19] to prevent genomic instability [20]. Some reports have been published on the 3D organization of telomeres in the nucleus, mainly with regard to the distances of telomeres from the nuclear shell. Telomeres have been previously found at the nuclear edge [21], at the nuclear periphery [22], throughout the entire nucleus [13,23], in non-Rabl association [11], in association with the nucleolus [24] or in the nuclear matrix [25].

Telomere dynamics also have been studied in living human U2OS osteosarcoma cells [26]. Individual telomeres showed significant directional movements and telomeres were shown to associate with promyelocytic leukemia bodies in a dynamic manner. This means that telomere structure is dynamic, and may be important for both transcriptional processes and for stabilizing chromosome positions in the nucleus.

We have developed a method of studying the organization of the genome by analysis of the 3D organization of telomeres in the nucleus and their positional changes along the cell cycle, using flow-sorted living cells. This method enables us to determine, for the first time, that telomere organization is cell-cycle dependent, with assembly of telomeres into a telomeric disk in the G2 phase. Moreover, we show for tumor cells that the 3D telomere organization is distorted and that telomeric aggregates are formed. These results emphasize a non-random and dynamic 3D nuclear telomeric organization and its importance to genomic stability.

Results and discussion

To study the organization and structure of the genome in the nucleus, we took the approach of labelling only the telomeres and measuring their 3D organization as indicators for chromosomal distribution. After the 3D fluorescent measurements, the data were analyzed with a programme that was developed for this study. The programme finds all the telomeres in the nucleus; their size, intensity and shape; and determines the telomeric organization inside the volume of the nucleus. One crucial property that we analyzed was the distribution of the telomeres inside the nuclear volume. We first segmented the nucleus and found the centre of each telomere. We then found the smallest convex set of polygons that contains all the telomeres (Fig. 1). This was done by using the Quickhull algorithm [27]. In most cases, we found that the volume contained by the telomeres resembles either a sphere or a flattened sphere (disk). It can be described as an ellipsoid with two similar radii ($a \approx b$) and a different third one (c; Fig. 2). Such a shape is called a spheroid. The level of flatness of the volume occupied by the telomeres can, therefore, be described by the ratio of the two radii that are different, a (or b) and $c - a/c$. The larger the ratio, the more oblate (or disk-like) is the shape of the volume occupied by the telomeres, while $a/c \approx 1$ means that the volume is spherical.

The optical resolution and signal-to-noise ratio are presented in Fig. 3. The images of two neighbouring telomeres that are 1200 nm and 400 nm apart, and the corresponding intensity along the line connecting the pair, indicates the smallest telomere distance that can still

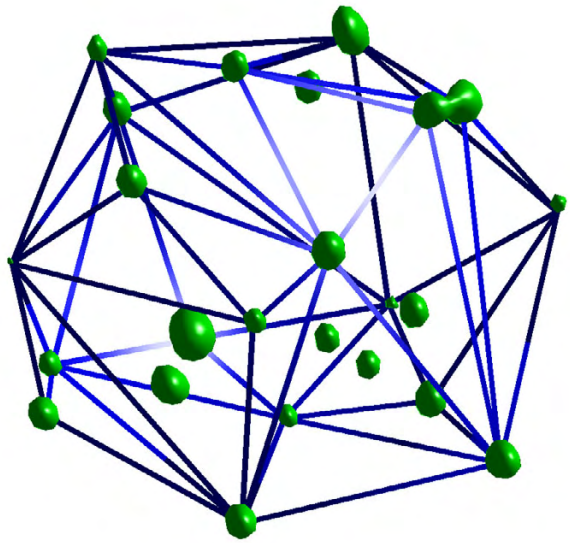

Figure 1
The distribution of the telomeres in the nucleus volume is found by fitting a convex set of polygons that contains all the telomeres. This volume usually looks like either a sphere or a disk and can be described as an ellipsoid.

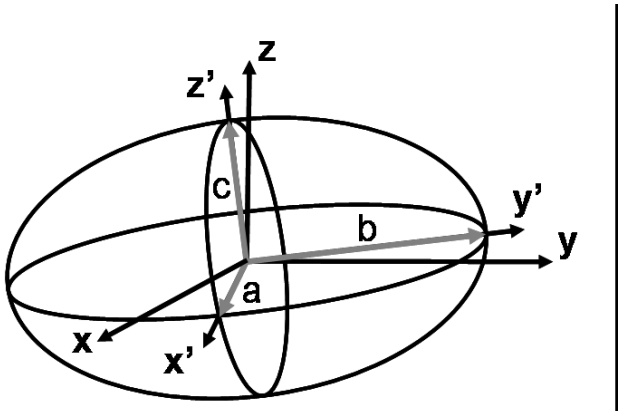

Figure 2
In general, the ellipsoid's main axes along *x'y'z'* do not coincide with the microscope-slide plane and optical axes *xyz*. Our programme finds an ellipsoid that contains all the telomeres and the size of its main axes *a*,*b*,*c*. In most of the cases the *x'y'* axes of the ellipsoid are similar, i.e. $a \approx b$. Therefore, the ratio *a*/*c* is a good measure of the flatness level of the ellipsoid and of the telomere organization inside the nucleus.

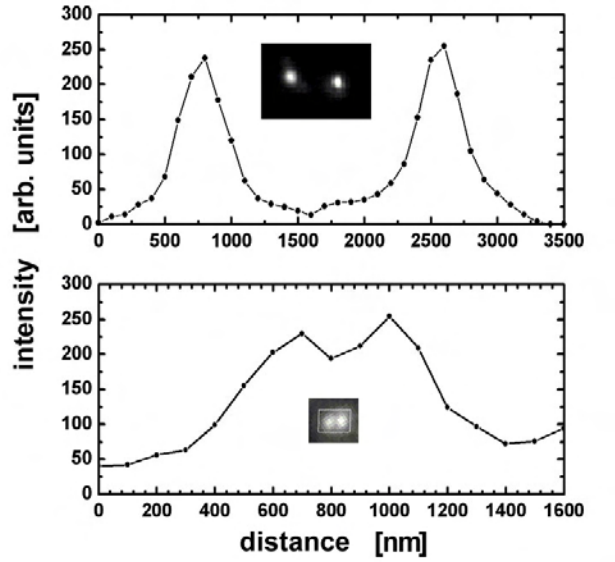

Figure 3
Demonstration of the signal-to-noise and spatial resolution of our measurements. The fluorescence intensity is bright (typical signal-to-noise ratio of 10:1). Two pairs of telomeres are shown, 1200 nm apart (top), which can be easily separated, and 400 nm apart (bottom). The inserts show the actual images.

be unambiguously distinguished (approximately 200 nm).

It is expected that 80 telomeres will be observed in the interphase nucleus for normal mouse cells (92 for a normal somatic human cell), however, in our measurements we were usually able to identify approximately 40 separated telomere regions in each mouse cell (50 in human cells). Similar results have been described before [23,28]. This is probably due to neighbouring telomeres that are closer than the optical resolution (see Fig. 3), but it does not affect the analysis of the telomere distribution in the nucleus as long as the hybridization efficiency is high. This was verified by two-dimensional measurements of all the telomeres in a metaphase spread (using the same probe), where at least 90% of the telomeres are unambiguously observed (Fig. 4).

We first described the major observation of primary BALB/c mouse B lymphocytes that were studied along the cell cycle. These studies were followed by the analysis of immortalized cells. The lymphocytes were sorted according to their DNA content for the determination of the G0/G1, S or G2/M phases (see *Methods*).

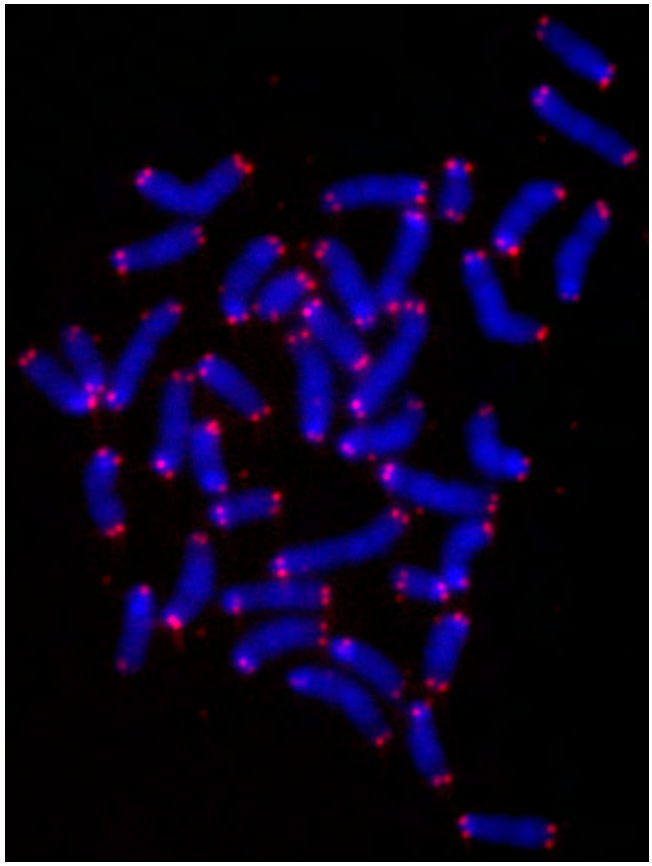

Figure 4
Metaphase plate prepared from fetal liver cells directly isolated from day 10 old mouse embryos. Metaphase chromosomes and spreads were prepared as described [30] and hybridized with a PNA-telomeric probe that was Cy3 labelled. More than 90% of the telomeres are clearly observed.

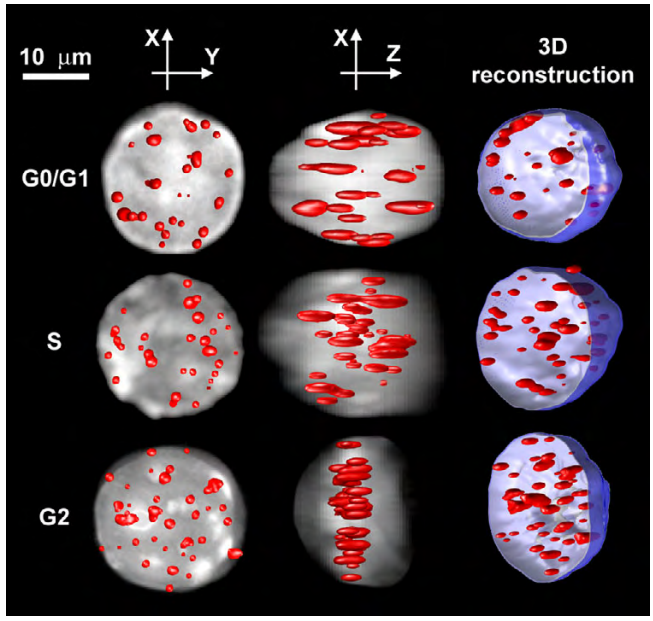

Figure 5
The distribution of telomeres in the nucleus of three typical cells selected from the G0/G1 phase (upper row), S phase (middle row) and G2/M phase (lower row). Each telomere distribution is shown from a top view (the *XY* plane), along the optical axis *Z* (left column), from a side view (*XZ* plane) as observed along the *Y* axis (centre column) and as a 3D image of the telomeres in an open nucleus (right column). When shown from the top and side views, the telomeres are displayed on top of the projected image of the nucleus. This projection demonstrates the extent of the chromatin (and therefore chromosomes) and defines the volume and borderline of the nucleus.

By analyzing cell-cycle sorted primary mouse lymphocytes we found that the 3D telomere organization changes during the cell cycle. Telomeres are widely distributed throughout the nucleus in the G0/G1 and S phases with a calculated *a*/*c* ratio of 0.9 ± 0.4, which means a spherical-like volume of distribution. However, during G2, telomeres are not observed throughout the whole nucleus. Their 3D organization changes, with all the telomeres assuming a central structure that we call the telomeric disk, which has never been reported before. In this ordered structure, all the telomeres align in the centre of the nucleus as cells progress into the late G2 phase. The *a*/*c* ratio they assume is 6.0 ± 2.0, which means a very flat disk (almost a coin shape).

Typical lymphocytes from different phases are shown in Fig. 5. The *a*/*c* ratio of these cells in the G0/G1, S and G2/M phases is 0.8, 0.8 and 6, respectively, and clearly shows the correlation of the *a*/*c* ratio with the telomere distribution and the organization of the telomeric disk that we found in the G2 phase. The elongation of the telomeres along the *Z* axis (the optical axis) relative to the *XY* plane has the same ratio as the point spread function of our system and results from the poorer optical resolution along the optical axis. However, this has a very small effect on the shape of the whole nucleus.

Similar results have been observed in primary human lymphocytes, primary human fibroblasts and in normal human epithelial tissue (see additional file for more data). This suggests that chromosomes assume a very precise order that pre-aligns them prior to the onset of mitosis. In order to ascertain that the telomeric disk was not the result of a distorted nucleus, our analysis programme

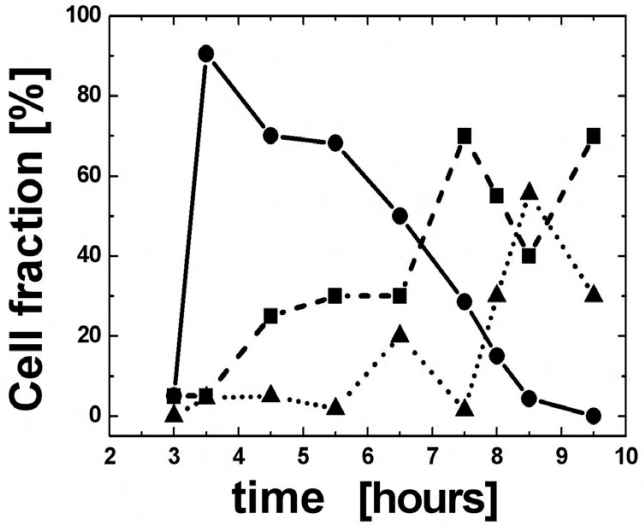

Figure 6
BrdU-positive cells were live sorted and synchronized in the S phase. They were harvested from a culture at time intervals of 3.5–9 hours. The cells were then fixed for 3D analysis. For each time point we have measured: 1. the fraction of nuclei with a telomeric disk; 2. the fraction of cells in mitosis; and 3. the fraction of cells with interphase nuclei but without a telomeric disk. Ninety percent of the cells formed a telomeric disk 3.5 hours after BrdU incorporation and were therefore interpreted as cells in the late G2 phase (black line and circles). Cells entering mitosis (dashed line and squares) peaked at 7.5 hours (65%) and cells in G1 (dotted line and triangles) peaked after 8.5 hours (57%). The increase in the number of metaphases at 9.5 hours cannot be explained and probably lies within the limits of experimental errors.

compared the telomere distribution volume and shape with that of the 4'-6-Diamidino-2-phenylindole (DAPI) – stained nucleus, and verified that the nucleus itself still had a spherical-like volume. We rarely found distorted nuclei and excluded these cells from the analysis. The nucleus shown in G2 is not fully spherical. Such a shape is expected, because when the telomeres forms a disk, it pools the chromosomes and forces them to be closer to the disk, which results in an oblate shape as well.

To further study the phase transition timing along the cell cycle we used the synchronous bromodeoxyuridine (BrdU) sorting method. The cell population was pulse-labelled with BrdU in the S phase and flow sorted. Cells were placed back into culture and sub-populations harvested at 3.5, 4, 5, 6, 7, 8, 8.5, 9 and 10 hours after labelling and sorting. The cells were then fixed for 3D analysis. A minimum of 20 cells from each of these sub-populations were measured, analyzed and divided into the following three categories: 1) nuclei with a telomeric disk; 2) cells in mitosis; 3) cells in interphase without telomeric disk and mitotic figures (evaluated as G1 cells). The cell fractions as a function of time are shown in Fig. 6. Most cells (90%) form a telomeric disk 3.5 hours after BrdU incorporation. These cells are, therefore, interpreted as cells in the G2 phase. The fraction of metaphase cells peaks at 7.5 hours (65%) and the cell fraction of interphase cells that does not have a telomeric disk (and is interpreted as being in the G1 phase) peaks at 8.5 hours (57%).

These results reveal that the telomeric disk is formed in the late G2 phase. As cells progress from G2 to M, chromosomes organize into metaphases and, therefore, the number of cells in interphase with a telomeric disk decreases. Because there is no other state of transition between telomeric disk and mitosis, we conclude that the telomeric disk is the 3D telomeric organization assumed in late G2. Thus, it is also the final stage of the interphase nucleus that permits the organization of the genetic material prior to its entry into the M phase and prior to chromosome segregation. Cells in late G2 with a telomeric disk have additional characteristic features: i) they exhibit a larger overall nuclear volume than their G1 or S phase counterparts (this increase in size was also confirmed by fluorescent activated cell sorter [FACS] analysis); and ii) they begin to show signs of early re-organization of the chromatin into partially condensed areas (as visualized using the DAPI stained image).

At the end of the M phase, we observe cells that enter into the G1 conformation of telomeres, with a wide spatial distribution of telomeres throughout a smaller nucleus.

In conclusion, this data indicates that the telomeric disk is a novel structure within the interphase nucleus in late G2 that has not been previously described. Its existence points to the fundamental importance of ordered nuclear organization at the end of G2. The telomeric disk probably assures the proper organization of chromosomes prior to mitosis and their organized segregation during mitosis. Together with information that has been previously published on telomeric dynamics [26,28], it is tempting to speculate that telomeres take an active part in the process of chromosome organization into a unique structure, the telomeric disk, during G2. This alignment of telomeres and chromosomes would facilitate the proper subsequent organization of the chromosomes into an equatorial plane during cell division. This process may be driven by the telomeres themselves (that are free of the nuclear matrix) or through the nuclear matrix. The telomeric disk may also allow for a late G2 checkpoint.

Further work on the subject can also be performed *in vivo*, as has been shown by Molenaar *et al.* [26]. In such a way the full dynamic process can be observed, which is complementary to the single time-points that are shown in our work.

We have continued to observe the distribution of telomeres in cancer cells. Typical 3D images constructed from normal nuclei and from a Burkitt lymphoma cell line (Raji), as well as from primary mouse plasmacytoma (PCT) and primary human head and neck squamous cell carcinoma (HNSCC) stage IV (Fig. 7), show that telomeres form aggregates and thus a partially altered telomeric disk. Such telomeric aggregates are characterized by both a larger volume and larger integrated intensity than their normal non-overlapping and non-aggregated counterparts. They are not observed in normal cells. Similar results for altered telomeric organization have also been found in human neuroblastoma and colon carcinoma tumor cell lines.

In line with these concepts, oncogenic activation remodels this nuclear order and sets the stage for genomic instability as we have recently measured for conditional c-Myc deregulation. We have found that deregulated expression of c-Myc alters the 3D nuclear organization of chromosomes and telomeres, and makes genomic rearrangements topologically feasible (Chuang *et al.*, in preparation).

Conclusions

In summary, we have shown that 3D optical imaging followed by the analysis of telomeres in the interphase is an important tool for basic research and cancer biology. We have found cell-cycle dependence of the telomere organization in the nucleus, where telomeres align into a telomeric disk during the late G2 phase. Such an organization has never before been reported.

Telomeric aggregates are found in tumor cells and, therefore, an alteration of the telomeric disk is seen. Transient telomeric aggregations potentially cause irreversible chromosomal rearrangements.

The above findings indicate that it is now possible to examine the presence of telomeric aggregates suggestive of genomic instability in individual interphase nuclei and tissue, without the need to examine metaphases. Such new directions of monitoring genomic instability could potentially have an impact on cancer biology, genetics, diagnostic innovations and surveillance of treatment response in medicine.

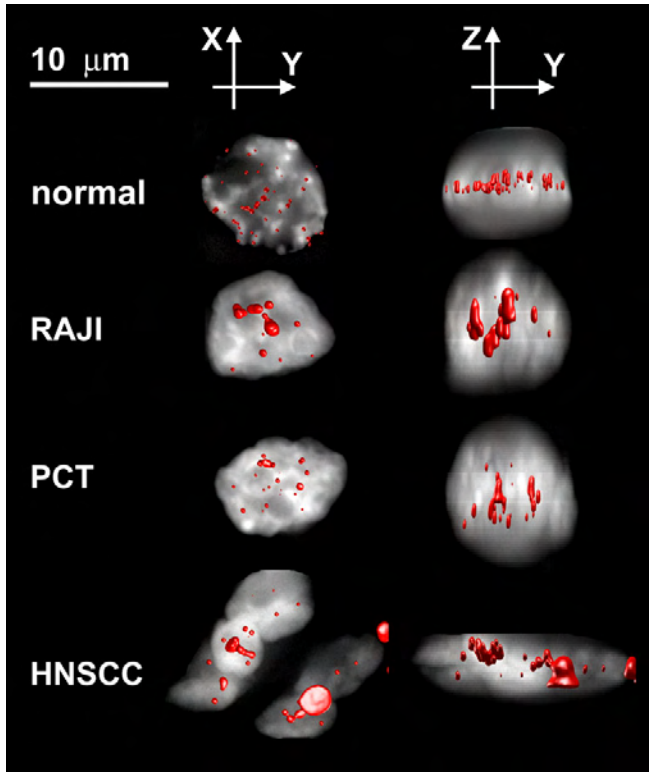

Figure 7
Normal: A normal blood cell; RAJI: A Burkitt lymphoma cell line; PCT: A primary mouse plasmacytoma cell; HNSCC: A primary human head and neck squamous cell carcinoma (stage IV). The distribution of telomeres in cancer cells compared with a normal cell. Images are shown as explained in Fig. 5. Aggregates of telomeres are formed and the telomere disk that appears in the G2 phase is distorted.

Methods

Cells

Mouse primary cells were directly isolated from BALB/c mice and stimulated with lipopolysaccharide to enter into the cell cycle [29]. Primary mouse fetal liver cells were also directly isolated from BALB/c mice. Mice were studied according to the protocols approved by Canadian Central Animal Care. Immortalized mouse pro B lymphocytes have been described elsewhere [30]. Human primary cells were obtained from healthy donors. Head and neck squamous cell carcinoma and control tissue were obtained from a patient at CancerCare Manitoba upon ethics approval and informed consent.

Fixation techniques

Pro B lymphocytes [30] were fixed in four ways: i) following cytospin preparations, cells were fixed in 3.7% formaldehyde (1×PBS/50 mM $MgCl_2$); ii) cells were allowed to

grow on glass slides and were fixed in 1% formaldehyde (3D fixation); iii) cells were fixed in suspension with 3.7% formaldehyde (3D fixation); and iv) cells were fixed in methanol:acetic acid (3:1) according to standard protocols [29]. Tissue was fixed following cryosection (5 μm sections were used) in 1% formaldehyde (1×PBS/50 mM $MgCl_2$). All hybridizations shown in this report were carried out after 3D fixation.

Fluorescent activated cell sorter (FACS) analysis

For FACS analysis, primary lymphocytes were fixed in 70% cold ethanol and stained with propidium iodide (1 μg/mL) following RNAse (20 μg/mL) digestion. The stained cells were analysed for DNA content by flow cytometry in a EPICS Altra cytometer (Beckman-Coulter). Cell cycle fractions were quantified with WinCycle software (Phoenix Flow Systems, San Diego, CA).

Cell sorting

Cells were stained with Hoechst 33342 (Molecular Probes) at a final concentration of 1 μg/mL for 90 minutes at 37°C and 5% of carbon dioxide (CO_2). Cells were sorted according to their DNA content (G0/G1, S and G2/M phases) with a EPICS Altra cytometer (Beckman-Coulter) equipped with a UV laser (Coherent, excitation at 350 nm) and a 460 nm band-pass filter.

BrdU labelling

Pro B lymphocytes were labelled *in vivo* with 10 μM of BrdU (5-Bromo-2'-deoxyuridine, SIGMA-ALDRICHT, Lyon, France) for one hour at 37°C in humidified atmosphere (5% CO_2). BrdU was then detected with 5 μL/1×106 cells of anti-BrdU-FITC (fluorescein isothiocyanate) antibody (TEBU, Le Perray-en-Yvelines, France) at identical conditions for 30 minutes. Thereafter, all BrdU (i.e. FITC)-positive cells were live sorted, placed into culture for different times and harvested at 3.5, 4, 5, 6, 7, 8, 8.5, 9 and 10 hours after labeling and sorting. The cells were then fixed for 3D analysis. For each time point we have measured: 1. the fraction of nuclei with a telomeric disk; 2. the fraction of cells in mitosis; and 3. the fraction of cells in interphase nuclei without telomeric disk and mitotic figures that were evaluated as G1 and S phase cells.

Telomere FISH using Cy3-labled PNA probes

Telomere FISH was performed as described [31] using a Cy3-labelled PNA probe (DAKO, Glostrup, Denmark). Telomere hybridizations were specific as shown by metaphase hybridizations and the correct number of the telomeric signals observed at the ends of chromosomes prepared from primary cells (Fig. 4).

3D image acquisition

Unless stated otherwise, 20–30 cells were analyzed by 3D imaging from each cell type and phase type. Part of the measurements were done with a confocal microscope (Leica AOBS-SP) and most of them with a conventional Axioplan 2 (Zeiss) with a cooled AxioCam HR CCD followed by deconvolution [30]. DAPI, FITC and Cy3 filters (Zeiss) were used in combination with Planapo 63×/1.4 oil (Zeiss). Axiovision 3.1 software with a deconvolution module and rendering module were used (Zeiss). Both methods gave similar results.

80–100 sections were acquired for each 3D nucleus, typically with 200 × 200 pixels per section with a ~100 × 100 nm nominal imaging area per pixel (steps of 200 nm along *Z*). The point-spread function of our system has a full width at half max of approximately 200 nm in the plane and 400 nm along the optical axis.

3D analysis of telomeres

In order to analyze the telomere distribution in the nucleus, we developed a special 3D image analysis programme. The main algorithmic part is described below. The programme (TeloView) is based on the Matlab computer language (The MathWorks, Natick, MA, USA) and some of the image processing algorithms are based on the DipImage library (developed at the Quantitative Imaging Group, Delft University of Technology, Delft, The Netherlands) [32].

The programme segments the nucleus volume by a derivative-based algorithm using a morphological top and bottom-hat algorithm [33]. The volume, intensity and centre of gravity are calculated for each spot. The programme then finds a principle plane in the nucleus ($x'y'$) that is the closest to all the telomeres (Fig. 2). This is especially important when a tissue section is analyzed, because this plane should not necessarily be parallel to the microscope slide plane.

The telomeric distribution inside the nucleus is described by fitting an ellipsoid to the volume occupied by the telomeres (three different main axes; Fig. 2). The distributions were found to be either oblate or spherical (i.e. the two principle axes along the main $x'y'$ plane of the spheroid are similar). It is, therefore, convenient to describe the distribution volume as a spheroid (i.e. an ellipsoid having two axes of equal length). As such, it is simpler to describe the spheroid degree of variation from a perfect sphere by the ratio a/c where a and b are the similar semi-axes and c is the third one. Such a description reflects the degree to which the telomere's volume is oblate.

Authors' contributions

TCYC performed the data analysis, wrote the discussion, performed the hybridizations, took all head and neck cancer samples from tumor collections during surgery to preparation of frozen sections, hybridization, analyses

and patient records. SMo performed some of the hybridizations and analyses while visiting SM's lab. YG developed the 3D analysis methods, 3D algorithms and the programme that analyses the nuclei, wrote the paper in its current version and acts as corresponding author. AYCC organized the data, tables, and G2 phase data. ITY, BV and RD took part in the development of the 3D analysis methods and algorithms, and the programme that analyses the nuclei. VM did the BrdU-labelling experiments and G2 study in France and in SM's lab. MP performed the G2 analysis in SM's lab. MB performed the metaphase telomere FISH. PDK directs the programme of head and neck surgery and provided some of the samples that were used for the study. TF supervised VM and MP in France. PB supervised SMo in Heidelberg. SM planned and carried out the project that was performed in SM's lab, and was supervisor for TCYC, SMo (while visiting SM's lab), AYCC, MB, MP and VM, and performed part of the experiments.

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1980 B.Sc. - Upper second class honours, Anatomy
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1984 -1985	House officer in Medicine and Surgery, Hackney and St. Bartholomew's Hospital, London

EDUCATION & TRAINING (cont'd)

1985-1986	Senior House Officer Emergency Medicine, Whittington Hospital, London
1986 - 1987	Rotating Senior House Officer Department of Medicine St. Mary's and St. Charles Hospitals, London
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1988 - 1989	Registrar in General and Respiratory Medicine Ealing Hospital, Middlesex
1989 - 1990	Registrar in Gastroenterology Hammersmith Hospital, London
1990 – 1994	Ph.D. *A molecular genetic analysis of ovarian cancer* Completed as an external student of the University of London, at the Imperial Cancer Research Fund (Internal: Galton Laboratory, UCL)

FELLOWSHIPS

1990 - 1994	Clinical Research Fellow Human Immunogenetics Laboratory Imperial Cancer Research Fund London and Honorary Research Fellow, Family Cancer Clinic - St. Mark's Hospital, London (affiliated with the Division of Medical and Molecular Genetics, KGT Medical School, University of London)

APPOINTMENTS

1994 -	Medical Scientist, Montreal General Hospital
1996- 2002	Assistant Professor, Department of Medicine, McGill University, Montreal.
1996- 2002	Assistant Professor, Department of Human Genetics, McGill University, Montreal
1996-	Senior Research Associate, Epidemiology Research Centre, Pavillon Hotel Dieu, Centre Hospitalier Université de Montréal (CHUM).
1996-	Project Director, Lady Davis Institute, Sir Mortimer B. Davis-Jewish General Hospital, Montreal
1996-	Assistant Physician, Montreal General Hospital
1996- Montreal	Assistant Physician, Royal Victoria Hospital,
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1998-	2002 Assistant Professor, Department of Oncology, McGill University, Montreal
2001-	Principal Investigator, Canadian Genetic Diseases Network
2001-	Director, Program in Cancer Genetics, Departments of Oncology and Human Genetics, McGill University
2001-	Vice-Chair, Genetic VRC, Canadian Cancer Etiology Research Network
2002-	Associate Professor (tenure), Departments of Medicine, Human Genetics and Oncology, McGill University, Montreal

AWARDS RECEIVED

1979: Junior Scholarship in Anatomy, Physiology and Biochemistry, St Bartholomew's Hospital.

1983: Health Education Council Elective Scholarship "Diabetes in China".

1990-1994: Clinical Research Fellow Bursary, Imperial Cancer Research Fund, London.

1994-1997: Fast Foundation Award of the Montreal General Hospital Research Institute.

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CURRENT COMPETITIVE GRANTS

Principal Investigator

Principal applicant: Foulkes, WD
CBCRA-IDEA: *BRCA1* splice variants and breast cancer risk: novel approaches using nanobiology.
($97,380 one year, 2006-2007)

Principal applicant: Foulkes, WD
Co-investigators: Bismar, T; Aloyz, R; Ghadirian, P
CBCRA: Toward the biological treatment of BRCA1-related breast cancer: EGF, EGFR and tyrosine kinase inhibitors
($413,834 over 3 years, 2006-2009)

Principal applicant: Foulkes, WD
Co-investigators: Nielsen, T; Mai, S
CBCRA: BRCA1, CDC4, Cyclin E, and chromsomal instability in breast cancer
($315,021 over 3 years, 2005-2008)

Co-investigator

Principal applicant: Mai, S
Co-investigators: Foulkes, WD; Watson, P

Susan G Komen Breast Cancer Foundation
The three-dimensional telomeric signature(s) of DCIS
(US $249,000 over three years 2006-2009)

Principal applicant: Isaacs, W
Co-applicants: Foulkes, WD; Epstein, J; Partin, A; Easton, D; Eeles, R; Maehle, L; Giles, G; Hopper, J; Whittemore, AS; Halpern, J; Hsieh, CL; Cussenot, O; Cancel, G; Jarvik, G; Bdzioch, M; Stanford, J; Ostrander, E; Schaid, D; Thibodeau, S; Gronberg, H; Cooney, K; Lange, E; Schleutker, J; Vogel, W; Cannon-Albright, L; Camp, N; Jianfeng Xu, Meyers, D.
NIH (USA): *Prostate cancer susceptibility: the ICPCG study.*
($US 228,000 as personal award over 4 years, 2002-2006)

Principal applicant: Batista, R
Co-applicants: Foulkes, WD; Blancquaert, I; Cleret de Langavant, G; Gaudet, D; Godard, B; Laflamme, N; Marcoux, A; Rousseau, F
CIHR: *Programme de recherche en appui aux politiques de santé en génétique dans un souci de qualité, d'effcience et de bien-être social.*
($ 1,000,000 over 4 years, 2003-2007, no financial award to WDF)

Principal applicant: Bismar, T
Co-applicants: Foulkes, WD; Rubin, M.A
Prostate Cancer Research Foundation of Canada (PCRFC): *Defining aggressive phenotype of prostate cancer using a multiplex of 12 gene model*
($ 60,000 over 2 years, 2005-2007)

Principal applicant: Narod, S
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CBCRI(Canada): *Risk factor analysis of hereditary breast and ovarian cancer*
($ 1,250,000 over 5 years, 2004-2009)

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Research : Survival following breast cancer in *BRCA1/2* mutation carriers

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Research: Genomic deletions in *MLH1* and MSH2
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STUDENTS

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Isabelle Thiffault, MSc student, 2002-2004:Towards a molecular understanding of proteus syndrome.
Current Position: PhD student, Université de Montréal.

Susan McVety, MSc student, 2003- 2005: Characterisation of cDNA deletions in *MLH1* and *MSH2*.
Current Position: Laboratory Technician.

Ioli Makriyianni, MSc student, 2003-2005: Mitochondrial and somatic mutations in hereditary breast cancer.

Tayma Khalil, MSc student 2005-: CDC4, cyclin E and hereditary breast cancer.

McGILL UNIVERSITY SUMMER STUDENTS
(2 month projects)

Tamar Flanders 1996. Project: Familial studies of colorectal and endometrial cancer*
Kevin Sanders 1996. Project: Familial risks of Thyroid Cancer and Breast/Thyroid cancer*
Nathalie Ng Cheong 1997. Project: *PTEN* mutations in familial cancer*
Marie-Noelle Hébert-Blouin 1998. Project: *GSTT1* and risk of head and neck cancer*
Nicola Matthews 1998. Project: Lobular breast cancer and familial cancer risk*
Karen Buzaglo 2000. Project: Familial factors in fallopian tube cancer*
Maral Ouzounian 2000. Project: Germ-line mutations in hereditary breast cancer
Annick Wong 2002. Project: Claudins and cancer*

*work published as a result of their project

McGILL UNIVERSITY INDEPENDENT STUDIES STUDENTS
(3—4 credits)

Kiersten Henderson 1999 Project: Association studies in thyroid cancer*
Ayesha Islam 1999 Project: BRCA1/2 mutations in pancreas cancer among French-Canadians
Elsa Lanke 1999 Project: Thyroid cancer/Gastric Cancer genetics*
Vanessa Rossigny 2003 Project: CHEK2 and breast cancer in the Ashkenazim
David Novak 2005 Project**:** CHEK2 and breast cancer in French Canadians

*work published as a result of their project

COMPLETED POST DOCTORAL FELLOWSHIPS

Ala-Eddin Moustafa PhD (1999-2002)
Research: Genetic factors in squamous cell carcinoma of the head and neck
Current position: Assistant Professor, Department of Oncology, McGill University

Long Qi Chen MD PhD (2004-2005)
Research: SNP Discovery in CHEK2
Current position: Professor of Cardiothoracic Surgery, Szechuan Province, China.

MEMBERSHIPS

1984	General Medical Council: registration number 2921080
1987	Royal College of Physicians (UK)
1996	Collège des Médecins du Quebec, licence number 96-449
2000	Association of Medical Geneticists of Québec (by examination)

PROFESSIONAL SOCIETIES
British Medical Association
British Society of Human Genetics
American Society of Human Genetics

McGILL UNIVERSITY DEPARTMENTAL COMMITTEES

2001-	Member, Curriculum Committee, Department of Human Genetics
2001-	Member, Fellowship Committee, Department of Human Genetics
2001-	Member, Standing Committee, Department of Human Genetics
2001-	Member, Management Committee, Department of Oncology

Ph.D. DEFENCE /M.Sc. REFEREE

PhD, McGill Dept. Biology

Ronald Lafreniere, June 17, 1997.

MSc, McGill Dept. Epidemiology and Statistics
Hela Makni, April 2000

MSc, McGill Dept. Biology
Sahar Sibani, January 2001

MSc, McGill Dept Epidemiology and Statistics
Nooshin Ahmadi Pour, January 2003

PhD, University of Toronto Faculty of Medicine
Alexander Liede, February 2003

PhD, McGill Dept. Experimental Medicine
Kevin Little, November 2004

MSc, University of Toronto, Faculty of Medicine
Sean Cleary, December 2004

INTERNATIONAL CONFERENCE ORGANISER

First International Symposium on Hereditary Breast and Ovarian Cancer, Montreal Oct 19-21, 2005. (www.odon.ca/brca/). Co-sponsored by the Program in Cancer Genetics and the Hereditary Breast and Ovarian Cancer Foundation (www.hboc.ca). Role: Scientific Director of Conference.

INTERNATIONAL COMMITTEES etc

Cancer Genetics Abstract Referee, ASHG meeting, San Francisco, CA, 1999.
Co-Chair, Breast Cancer Genetics Session, ASHG, Denver, CO, 1998.
Member, Steering Committee, International Prostate Cancer Genetics Collaborative Group (representing Eastern Canada) 1997-
Writing committee, Cancer Genetics Certification Examination, Institute for Clinical Evaluation, American Board of Internal Medicine, Philadelphia, PA 1999-2000
Scientific Organising Committee, UICC International Conference on Familial Cancer, Oklahoma City, OK, June 4-6, 2003.

NATIONAL and INTERNATIONAL PEER-REVIEW GRANT COMMITTEE etc

National Cancer Institute of Cancer, Epidemiology panel, 1997-2000
Canadian Breast Cancer Research Initiative, IDEA grant panel, 2002-2003
Canadian Institute for Health Research, Genetics Panel, 2003-
ad hoc external reviewer of grants for MRC (Canada) (6), Alberta Heritage Fund for Medical Research (1), Cancer Research Campaign (UK) (5) Research Grants Council of Hong Kong (3), Yorkshire Cancer Research (1).

Tenure review, Independent Investigator, National Human Genome Research Institute, January 2001.
Promotion review (to Assistant Professor) Memorial Sloan-Kettering Cancer Center, June 2001.
Tenure review (to Associate Professor), University of Vermont, September 2002
Tenure review (to Full Professor) Memorial Sloan-Kettering Cancer Center, January 2003
Tenure review (to Full Professor) Sloan Kettering Institute and Memorial Sloan-Kettering Cancer Center, January 2003
Promotion review (to Clinical Assistant Professor), Ohio State University, July 2003
Promotion review (to Reader), University of London, May 2004
Promotion review (to Clinical Assistant Professor), Ohio State University, August 2004
Promotion review (to Clinical Assistant Professor), Ohio State University, August 2004
Promotion review (to Clinical Associate Professor), Ohio State University, April 2005
Promotion review (to Professor), University of London, April 2005

PROVINCIAL EXPERT COMMITTEE

Member, Advisory Board, Conseil d'Évaluation des technologies de la santé du Québec, 1999-

NIH CANCER WORKSHOP

Invited attendee, NCI/NIDCFR/NIDCD Head and Neck Cancer Workshop, Bethesda, Maryland, February 21-23, 1999

VISITING LECTURESHIP

Samuel Riven Lectureship, Vanderbilt University, Tennessee, USA, September 1-3, 1999.

TEACHING

a) NATIONAL
Workshop on the Genetics of Cancer, Royal College of Physicians of Canada, Montreal, September 23, 1999.

b) McGILL UNIVERSITY
1) Environmental Carcinogenesis (516-614B MSc program) 1996-1999, 2003, 2005
(One two-hour session)

2) Unit 8 small group teaching in medical genetics (medical students) 1997-
(One 2 hour lecture and 4 small group sessions, 3 hours each)

3) Genetics course (biology BSc students) 1998-1999
(Six one hour lectures and one 2 hour pre-exam session)

4) Unit 1 teaching (medical students) 2001-
(one two hour seminar)

5) Special Topics in Epidemiology and Biostatistics: Introduction to genetic epidemiology and statistical methods for human genetics (513-670A, Department of Epidemiology and Biostatistics) (2001-2, one 1 hour seminar)

6) ICM whole class oncology teaching: Three lectures on the prevention of Colorectal cancer (2002-2004)

7) Experimental and Clinical Oncology #5160635D: Cancer Genetics-1.5 hour seminar (2002-)

8) Inherited Cancer Syndromes, 521-690B, Department of Human Genetics: Four 2 hour lectures (2003-)

c) HOSPITAL

1) Genetics in Oncology Lecture (Residents) 1998-1999
(1.5 hour teaching session residents, SMBD-Jewish General Hospital)
2) Hereditary Breast Cancer (Surgical Residents) November 2005
(1 hour teaching session surgical residents, RVH)
3) Hereditary Breast Cancer (Surgical Residents) October 2006
(1 hour teaching session surgical residents, RVH)

HOSPITAL COMMITTEE

Montreal General Hospital Research Ethics Committee member, 1998-2004

PUBLICATIONS (* denotes WF corresponding author if more than one author)

a) Peer Reviewed Articles

1. Levi S, Beardshall K, Swift I, Foulkes WD, Playford R, Ghosh P and Calam, J: Antral Helicobacter pylori, hypergastrinaemia, and duodenal ulcers: effect of eradicating the organism. *Br Med J*, 299: 1504-5, 1989.
2. Campbell IG, Jones T, Foulkes WD, and Trowsdale J: Folate Binding Protein is a marker for ovarian cancer. *Cancer Res,* 51: 5529-5538, 1991.
3. Campbell IG, Freemont PS, Foulkes WD, and Trowsdale J: An ovarian tumour marker with homology to vaccinia virus contains an IgV-like region and multiple transmembrane domains. *Cancer Res,* 52: 5416-5420, 1992.
4. Foulkes WD, Campbell IG, Stamp GWH and Trowsdale J: Loss of heterozygosity and amplification of chromosome 11 in human ovarian cancer. *Br J Cancer*, 67: 268-273, 1993.
5. Foulkes WD, Ragoussis J, Stamp GWH, Allan GJ and Trowsdale J: Frequent loss of heterozygosity on chromosome 6 in human ovarian carcinoma. *Br J Cancer*, 67: 551-559, 1993.

6. Foulkes WD, Black DM, Stamp GWH, Solomon E and Trowsdale J: Very frequent loss of heterozygosity on chromosome 17 in ovarian carcinoma. *Int J Cancer*, 54: 220-225, 1993.

7. Naylor MS, Stamp GWH, Foulkes WD, Eccles DM and Balkwill FR: Tumor Necrosis Factor and its receptors in human ovarian cancer-potential role in disease progression *J Clin Invest*, 91: 2194-2206, 1993.

8. Allan GJ, Cottrell S, Trowsdale J and Foulkes WD: Loss of heterozygosity on chromosome 5 in sporadic ovarian carcinoma is a late event and is not associated with mutations in *APC* at 5q21-22. *Human Mutation*, 3: 283-291, 1994.

9. Foulkes WD, Englefield P and Campbell IG: Mutation analysis of *RASK* and the 'FLR exon' of *NF1* in sporadic ovarian carcinoma. *Eur J Cancer,* 30A: 528-530, 1994.

10. Campbell IG, Nicolai HM, Foulkes WD, Stamp GWH, Senger G, Allan GJ, Boyer CM, Jones K, Bast RC Jr, Solomon E, Trowsdale J and Black DM: A novel gene encoding a B-Box protein within the BRCA1 region at 17q21.1. *Hum Mol Genet,* 3: 589-594, 1994.

11. Campbell IG, Foulkes WD, Senger G, Trowsdale J, Garin-Chesa P and Rettig WJ: Molecular cloning of the B-CAM cell surface glycoprotein of epithelial cancers: a novel member of the immunoglobulin super-family. *Cancer Res,* 54: 5761-5765, 1994.

12. Englefield P, Foulkes WD and Campbell IG: Loss of heterozygosity on chromosome 22q in ovarian carcinoma is distal to and is not accompanied by mutations in *NF2* at 22q12. *Br J Cancer,* 70: 905-907, 1994.

13. Foulkes WD, Stamp GWH, Afzal S, Lalani N, McFarlane C, Trowsdale J and Campbell IG: *MDM2* amplification is uncommon in ovarian carcinoma irrespective of *TP53* status. *Br J Cancer,* 72: 883-8, 1995.

14. Foulkes WD, Brunet J-S, Kowalski LP, Narod SA and Franco EL: Family history is a risk factor for squamous carcinoma of the head and neck in Brazil: a case-control study. *Int J Cancer,* 63: 769-773, 1995.

15. Campbell, Foulkes WD, Beynon G, Davis M and Englefield P: LOH and mutation analysis of *CDKN2* in primary human ovarian cancers. *Int J Cancer,* 63: 222-225, 1995.

16. Phelan CM, Lancaster JM, Tonin P, Gumbs C, Carter R, Ghadirian P, Perret C, Moslehi R, Dion F, Faucher M-C, Dole K, Karimi S, Foulkes WD, Lounis H, Warner E, Goss P, Anderson D, Larsson C, Narod SA and Futreal PA: Mutation analysis of the BRCA2 gene in 49 site-specific breast cancer families. *Nature Genetics,* 13:121-123, 1996.

17. Afzal S, Lalani EN, Foulkes WD, Boyce B, Tickle S, Cardillo MR, Baker T, Pignatelli M and Stamp GWH: Matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 espression and synthetic matrix metalloproteinase-2 inhibitor binding in ovarian carcinomas and tumour cell lines. *Laboratory Invest,* 74: 406-421, 1996.

18. Bryan EJ, Watson RH, Davis M, Hitchcock A, Foulkes WD and Campbell IG: Localization of an ovarian cancer tumor suppressor gene to a 0.5cM region between D22S284 and CYP2D, on chromosome 22q. *Cancer Res,* 56: 719-721, 1996.

19. Davis M, Hitchcock A, Foulkes WD and Campbell IG: Refinement of two chromosome 11q regions of heterozygosity in ovarian cancer. *Cancer Res,* 56: 741-744, 1996.

20. Foulkes WD, Bolduc N, Lambert D, Ginsburg O, Yandell DW, Tonin P and Narod SA: Increased incidence of cancer in first degree relatives of women with double primary carcinomas of the breast and colon. *J Med Genet,* 33: 534-539, 1996.

21. Foulkes WD, Brunet J-S, Sieh W, Shenouda G, Black MJ and Narod SA: Familial risks of squamous cell carcinoma of the head and neck. *Br Med J,* 313: 716-721, 1996.

22. Tonin P, Weber B, Offit K, Couch F, Rebbeck TR, Neuhausen S, Godwin AK, Daly M, Wagner-Costalos J, Berman D, Grana G, Fox E, Kane MF, Kolodner RD, Krainer M, Haber DA, Struewing JP, Warner E, Rosen B, Lerman C, Peshkin B, Norton L, Serova O, Foulkes WD, Lynch HT, Lenoir GM, Narod SA and Garber JE: Frequency of recurrent BRCA1 and BRCA2 mutations in Ashkenazi Jewish breast cancer families. *Nature Med,* 2:1179 -1183, 1996.

23. Milner BJ, Hosking L, Sun S, Haites NE, Foulkes WD: Polymorphisms in $P21^{CIP1/WAF1}$ are not correlated with TP53 status in sporadic ovarian tumours. *Eur J Cancer*, 32A: 2360-2363, 1996.

24. Cutler C, Foulkes WD, Brunet J-S, Flanders T, Shibata H and Narod SA: Cutaneous malignant melanoma is uncommonly associated with a family history of melanoma. *Melanoma Res,* 6: 435-440, 1996.

25. Foulkes WD, Buu PN, Filiatrault D, Leclerc J-M and Narod SA: An excess of congenital abnormalities in French-Canadian children with neuroblastoma: A case series study from Montreal. *Med Pediatr Oncol,* 29: 272-79, 1997

26. Karp SE, Tonin PN, Bégin LR, Martinez JJ, Zhang JC, Pollak MN and Foulkes WD: Influence of BRCA1 mutations on nuclear grade and estrogen receptor status on breast carcinoma in Ashkenazi Jewish women. *Cancer,* 80: 435-441, 1997.

27. Foulkes WD, Wong N, Brunet J-S, Narod SA, Bégin LR, Zhang JC, Martinez JJ, Tonin PN, Karp SE, Pollak MN. Germ-line *BRCA1* mutation is an adverse prognostic factor in Ashkenazi Jewish women with breast cancer. *Clin Cancer Res,* 3: 2465-2470, 1997

28. Sun S, Pollock P, Liu L, Karimi S, Jothy S, Milner BJ, Renwick A, Lassam NJ, Hayward NK, Hogg D, Narod SA and Foulkes WD: *CDKN2A* mutation in a non-FAMMM kindred with cancers at multiple sites results in a functionally abnormal protein. *Int J Cancer,* 73: 531-536, 1997.

29. Bignell GR, Canzian F, Shayeghi M, Stark M, Shugart YY, Biggs P, Mangion J, Hamoudi R, Rosenblatt J, Buu P, Sun S, Stoffer SS, Goldgar DE, Romeo G, Houlston RS, Narod SA, Stratton MR and Foulkes WD: A familial non-toxic multinodular thyroid goiter locus maps to chromosome 14q but does not account for familial non-medullary thyroid cancer. *Am J Hum Genet,* 61: 1123-1130, 1997.

30. Eeles RA, Simard J, Teare D, Edwards S, Durocher F, Badzioch M, Hamoudi R, Gill S, Biggs P, Dearnaley D, Arden-Jones A, Dowe A, Shearer R, Ford D, Amos C, The CRC/BPG Familial Prostate Cancer Study Collaborators, Ghadirian P, Aprikian A, Norman R, McLellan D, Labrie F, Narod SA, Easton D and Foulkes WD: Linkage analysis of chromosome 1q markers in 136 prostate cancer families. *Am J Hum Genet,* 62: 653-658, 1998.

31. Brunet J-S, Ghadirian P, Rebbeck TR, Lerman CE, Garber JE, Tonin PN, Abrahamson J,

    Foulkes WD, Daly M, Wagner-Costalas J, Godwin A, Olopade F, Moslehi R, Liede A, Futreal PA, Weber B, Lenoir GM, Lynch HT and Narod SA: The effect of smoking on breast incidence in BRCA1 and BRCA2 carriers. *J Natl Canc Inst,* 90: 761-766, 1998.

32. Pollock PM, Spurr N, Bishop DT, Newton-Bishop J, Gruis N, Goldstein AM, Tucker MA, Foulkes WD, Barnhill R, Haber D, Fountain J and Hayward NK: Haplotype analysis of 2 recurrent CDKN2A mutations in 10 melanoma families from three continents-evidence for common founders. *Hum Mutation,* 11: 424-431, 1998.

33. Godard B, Foulkes WD, Provencher D, Brunet J-S, Tonin PN, Mes-Masson, A-M, Narod SA and Ghadirian P: Risk factors for familial and sporadic ovarian cancer among French-Canadians: a case-control study. *Am J Obs Gyne,* 179: 403-410, 1998.

34. Kerr B, Foulkes WD, Cade D, Hadfield L, Hopwood P, Serruya C, Hoare F, Narod SA and Evans DG: False family history of breast cancer in the family cancer clinic. *Eur J Surg Oncol,* 24: 275-279, 1998.

35. Tonin PN, Mes-Masson A-M, Futreal PA, Morgan K, Mahon M, Foulkes WD, Cole DEC, Provencher D, Ghadirian P and Narod SA: Founder *BRCA1* and *BRCA2* mutations in French Canadian breast and ovarian cancer families. *Am J Hum Genet,* 63: 1341-1351, 1998.

36. Pal T, Flanders T, Lehman M, McMillan A, Brunet J-S, Narod SA and Foulkes, WD: Genetic implications of double primary cancers of the colorectum and endometrium *J Med Genet,* 35: 978-984, 1998.

37. de Andrade M, Amos, CI and Foulkes WD: Segregation analysis of squamous cell carcinoma of the head and neck: evidence for a major gene determining risk. *Ann Hum Genet,* 62: 505-510, 1998.

38. Wang Z-J, Churchman M, Campbell IG, Xu W-H, Yan Z-Y, McCluggage WG, Foulkes WD and Tomlinson IPM: Allele loss and mutation screen at the Peutz-Jeghers (*LKB1*) locus (19p13.3) in sporadic ovarian tumours. *Br J Cancer,* 80: 70-72, 1999.

39. Pereira P, Stanton V, Jothy S, Tomlinson IPM, Foulkes WD and Rozen R: Loss of heterozygosity of methylenetetrahydrofolate reductase in colon carcinomas. *Oncology Reports,* 6: 597-599, 1999.

40. Warner E, Foulkes WD, Goodwin P, Meschino W, Blondal J, Patterson C, Ozcelik H, Goss P, Allingham-Hawkins D, Hamel N, Di Prospero L, Contiga V, Serruya C, Klein M, Moslehi R, Honeyford J, Liede A, Glendon G, Brunet J-S and Narod S: Prevalence and penetrance of BRCA1 and BRCA2 mutations in unselected

Ashkenazi Jewish women with breast cancer *J Natl Cancer Inst*, 91: 1241-1247, 1999.

41. Breast Cancer Linkage Consortium: Cancer risks in *BRCA2* mutation carriers. *J. Natl Cancer Instit*, 91: 1310-1316, 1999 (WDF was one of the many who contributed data to this publication).

42. Yuan ZQ, Bégin LR, Wong N, Brunet J-S, Trifiro M, Gordon PH, Pinsky L and Foulkes WD: The effect of the I1307K *APC* polymorphism on the clinicopathological features and natural history of breast cancer. *Br J Cancer*, 81: 850-854, 1999.

43. Jernström H, Lerman C, Ghadirian P, Lynch H, Weber B, Garber J, Daly M, Olopade O, Foulkes WD, Warner E, Brunet J-S and Narod SA. Pregnancy increases the risk of early breast cancer in *BRCA1* and *BRCA2* carriers. *Lancet*, 354:1846-1850, 1999.

44. Hamel N, Black MJ, Ghadirian P and Foulkes WD: No association between *p53* codon 72 polymorphism and risk of squamous cell carcinoma of the head and neck. *Br J Cancer*, 82: 757-759, 2000.

45. Xu JF and International Consortium for Prostate Cancer Genetics (ICPCG). Combined Analysis of Hereditary Prostate Cancer Linkage to *1q24-25*: Results from 772 Hereditary Prostate Cancer Families from the International Consortium for Prostate Cancer Genetics. *Am J Hum Genet*, 66: 945-957, 2000 (WDF is a member of the ICPCG writing committee for this paper).

46. Foulkes WD, Chappuis PO, Wong N, Brunet J-S, Vesprini D, Rozen F, Yuan ZQ, Pollak MN, Kuperstein G, Narod SA and Bégin LR. Primary node negative breast cancer in *BRCA1* mutation carriers has a poor outcome. *Ann Oncol*, 11: 307-313, 2000.

47. Rahman N, Arbour L, Houlston R, Bonaïti-Pellié C, Abidi F, Tranchemontagne J, Ford D, Narod SA, Pritchard-Jones K, Foulkes WD, Schwartz C and Stratton MR. Penetrance of mutations in the familial Wilms tumor gene, *FWT1*. *J Natl Cancer Inst*, 92: 650-652, 2000.

48. Liu G, Ghadirian P, Vesprini, D, Hamel N, Paradis A-J, Lal G, Gallinger S, Narod SA and Foulkes WD. Polymorphisms in *GSTM1*, *GSTT1* and *CYP1A1* and risk of pancreatic cancer. *Br J Cancer*, 82: 1646-9, 2000.

49. Kuperstein G, Foulkes WD, Ghadirian P, Hakimi J and Narod SA. A rapid fluorescent multiplexed-PCR analysis (FMPA) for founder mutations in the *BRCA1* and *BRCA2* genes *Clin Genet*, 57: 213-220, 2000.

50. Rapley E, Barfoot R, Bonaiti-Pellie C, Chompret A, Foulkes WD, Perusinghe N, Reeve A, Royer-Pokora B, Schumacher V, Shelling A, Skeen J, de Tourreil S, Weirich A, Pritchard-Jones K, Stratton MR, Rahman N. Evidence for susceptibility genes to familial Wilms tumour in addition to *WT1*, *FWT1*and *FWT2*. *Br J Cancer*, 83: 177-83, 2000.

51. Yuan ZQ, Chong GL, Thompson-May S, Trifiro M, Khandjian EW, Rousseau F, Yandell D, McNamara E, Gordon PH and Foulkes WD. Analysis of the hMLH1 and hMSH2 genes in three Quebec families with Hereditary Non-Polyposis Colorectal Cancer. *Ann Roy Coll Phys Surg Canada*, 33: 287-291, 2000.

52. Metcalfe K, Liede A, Hoodfar E, Scott A, Foulkes WD and Narod SA: An evaluation of needs among female *BRCA1* and *BRCA2* mutation carriers undergoing genetic counselling. *J Med Genet,* 37: 866-874, 2000.

53. Chappuis PO, Kapusta L, Bégin LR, Wong N, Brunet J-S, Narod SA, Slingerland J and Foulkes WD: Germline *BRCA1/2* mutations and $p27^{Kip1}$ protein levels independently predict outcome after breast cancer. *J Clin Oncol,* 18: 4045-4052, 2000.

54. Thompson D, Easton D and the Breast Cancer Linkage Consortium. Variations in Cancer Risks by mutation position in BRCA2 mutation carriers. *Am J Hum Genet,* 68: 410-9, 2001. (WDF was one of the many who contributed data to this publication).

55. Tonin PN, Perret C, Lambert J, Paradis A-J, Kantemiroff T, Benoit M-H, Martin B, Foulkes WD, Ghadirian P. Founder *BRCA1* and *BRCA2* mutations in early-onset French Canadian breast cancer cases unselected for family history *Int. J Cancer,* 95: 189-193, 2001.

56. Manning AP, Abelovich D, Ghadirian P, Lambert JA, Frappier D, Provencher D, Robidoux A, Peretz T, Narod SA, Mes-Masson A-M, Foulkes WD, Wang T, Morgan K, TM Fujiwara, Tonin PN. Haplotype analysis of BRCA2 8765delAG mutation carriers in French Canadian and Yemenite Jewish Hereditary Breast Cancer Families. *Human Heredity,* 52: 116-120, 2001.

57. Pal T, Hamel N, Vesprini D, Sanders K, Mitchell M, Quercia N, Ng Cheong N, Murray A, Foulkes WD and Narod SA. Double primary cancers of the breast and thyroid in women: molecular analysis and genetic implications. *Familial Cancer,* 1: 17-24, 2001.

58. Satagopan JM, Offit K, Foulkes WD, Robson ME, Wacholder S, Eng CM, Karp SE, Begg CB. The lifetime risk of breast cancer in Ashkenazi Jewish carriers of *BRCA1* and *BRCA2* mutations. *Cancer Epidemiology Biomarkers and Prevention,* 10: 467-73, 2001.

59. Greenblatt MS, Chappuis PO, Bond JP, Hamel N and Foulkes WD. TP53 mutations in BRCA1/2-related breast cancer: distinctive spectrum and structural distribution. Cancer Research, 61: 4092-97, 2001.

60. Runnebaum IB, Wang-Gohrke S, Vesprini D, Kreienberg R, Lynch H, Moslehi R, Ghadirian P, Weber B, Godwin AK, Risch H, Garber J, Lerman C, Olapade OI, Foulkes WD, Karlan B, Warner E, Rosen B, Rebbeck T, Tonin P, Dubé M-P, Kieback DG, Narod SA. Progesterone receptor variant increases ovarian cancer risk in BRCA1 and BRCA2 mutation carriers who were never exposed to oral contraceptives. Pharmacogenetics, 11: 1-4, 2001.

61. Chappuis PO, Hamel N, Paradis A-J, Deschênes J, Robidoux A, Potvin C, Cantin J, Tonin PN, Ghadirian P, Foulkes WD. Prevalence of founder BRCA1 and BRCA2 mutations in unselected French Canadian women with breast cancer. Clin Genet, 59: 418-423, 2001.

62. Bevan S, Pal T, Greenberg CR, Green H, Wixey J, Bignell G, Narod SA, Foulkes WD, Stratton MR and Houlston RS. A comprehensive analysis of MNG1, TCO1,

fPTC, PTEN, RET, TSHR and TRKA in Familial Non-Medullary Thyroid Cancer: Confirmation of linkage to TCO1. J Clin Endocrinol Metab, 86: 3701-4, 2001.

63. Julian-Reynier C, Bouchard L, Evans DGR, Eisinger F, Foulkes WD, Kerr B, Blanquaert, Moatti J-P and Sobol H. Women's attitudes towards preventive strategies for Hereditary Breast/Ovarian cancer risk differ from one country to another: differences between Manchester (UK), Marseilles (F) and Montreal (Ca). Cancer, 92: 959-68, 2001.

64. Elhaji YA, Gottleib B, Lumbroso R, Beitel LK, Foulkes WD, Pinsky L and Trifiro M. The Polymorphic CAG Repeat of the Androgen Receptor Gene: A Potential Role in Breast Cancer in Women over 40. *Breast Cancer Research and Treatment,* 70:109-16, 2001.

65. Pal T, Vogl FD, Chappuis PO, Tsang R, Brierley J, Renard H, Sanders K, Kantemiroff T, Bagha S, Goldgar DE, Narod SA and Foulkes WD. Increased risk for non-medullary thyroid cancer (NMTC) in the first-degree relatives of prevalent cases of NMTC: a hospital-based study. J Clin Endocrinol Metab, 86: 5307-12, 2001

66. Jefferies S, Edwards SM, Hamoudi R, A'Hern R, Foulkes WD, Goldgar D, MPT collaborators and Eeles R. No germline mutations in CDKN2A (p16) in patients with squamous cell cancer of the head and neck and second primary tumours. Br J Cancer, 85: 1383-6, 2001.

67. Yuan ZQ, Gottlieb B, Beitel LK, Wong N, Gordon PH, Wang Q, Puisieux, A, Foulkes WD and Trifiro M. Polymorphisms and HNPCC: PMS2-MLH1 protein interactions diminished by 'single nucleotide polymorphisms'. Human Mutation, 19: 108-13, 2002.

68. Ghadirian P, Liu G, Gallinger S, Schmocker B, Paradis A-J, Lal G, Brunet J-S, Foulkes WD and Narod SA. Family history is a risk factor for pancreatic cancer. Int J Cancer, 97: 807-810, 2002.

69. Moustafa A-E, Alaoui-Jamali MA, Batist G, Hernadez-Perez M, Serruya C, Alpert L, Black MJ, Sladek R and Foulkes WD. Identification of genes associated with head and neck carcinogenesis by cDNA microarray comparison between matched normal and squamous cell carcinoma cells. Oncogene, 21: 2634-2640, 2002.

70. Hutter P, Wijnen J, Rey-Berthod C, Thiffault I, Verkuijlen P, Farber D,Hamel N, Bapat B, Thibodeau SN, Burn J, Wu J, MacNamara E, Heinimann K, Chong G and Foulkes WD. An MLH1 haplotype is over-represented on chromosomes carrying an HNPCC predisposing mutation in MLH1. J Med Genet, 39: 323-327, 2002.

71. Laazari K, Sutton M, Ghadirian P, Scott A, Paradis A-J, Tonin PN and Foulkes WD. Is there are correlation between the structure of hair and breast cancer or BRCA1/2 mutations? Phys Med Biol, 47: 1623-1632, 2002.

72. Foulkes WD, Thiffault I, Gruber SB, Horwitz M, Hamel N, Lee C, Shia J, Markowitz A, Figer A, Friedman E, Farber D, Greenwood CMT, Bonner JD, Walsh T, Marcus V, Tomsho L, Nafa K, Gebert J, Bressac-de Paillerets B, Gregersen PK, Weitzel J, Macrae FA, Gaff CL, Gordon PH, MacNamara E, King M-C, Hampel H, de la Chapelle A, Boyd J, Offit K, Rennert G, Chong G and Ellis NA. The founder mutation MSH2*1906G>C is an important cause of Hereditary Non-Polyposis

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35. Julian-Reynier CM, Evans G, Bouchard L, Eisinger F, Kerr B, Foulkes W, Blancquaert I, Sobol H. Consultees' attitudes towards preventive strategies for Hereditary Breast Ovarian Cancer risk: Cultural differences between Manchester (UK), Marseilles (F) and Montreal (Ca). Am J Hum Genet 65S: A12, 58, 1999

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37. Chappuis PO, Renard H, Kantemiroff T, Sanders K, MacMillan A, Black MJ, Goldgar DE and Foulkes WD. Increased incidence of thyroid cancer in the first degree relatives of probands with non-medullary thyroid cancer: a case-control-cohort study. Am J Hum Genet A121, 643, 1999

38. Kasprzak L, Nolet S, Gaboury L, Gregory W, Pavia C, Villabona C, Rivera-Fillat, Oriola J and Foulkes WD. Familial medullary thyroid carcinoma and prominent corneal nerves associated with a codon 804 germline mutation of the RET gene. Am J Hum Genet A132, 706, 1999

39. Foulkes WD, Satagopan JM, Chappuis PO, Wong N, Bégin LR, Boyd J, Offit K and Robson ME. The presence of a germ-line BRCA mutation is an independent poor prognostic marker in breast cancer: a two-center historical cohort study. Am J Hum Genet A105, 379, 2000

40. Robson ME, Roberge D, Chappuis PO, Offit K and Foulkes WD. Risk of Ipsilateral Recurrence (IBTR) and Metachronous Contralateral Breast Cancer (CBC) after Breast Conservation Therapy (BCT) in Women with Germline *BRCA1* or *BRCA2* Mutations. Slide presentation at the 23rd Annual San Antonio Breast Cancer Symposium. Presented in General Session IV, December 8, 2000.

41. Roberge D, Foulkes W, Wong N, Chappuis P, Bégin L, Chow T. Résultats de la mastectomie partielle suivie de radiothérapie chez les porteuses de gènes *BRCA1/BRCA2* mutants: implications thérapeutiques. Oral communication, 11e congrès national de la société française de radiothérapie oncologique, Paris, 23-24 novembre 2000. Cancer Radiothérapie 4: 1s 10s, 2000.

42. Canil CM, Markin G, Chappuis P, Goshen R, Meschino W, Goss P, Narod S, Foulkes W, Warner E. Comparison of clinical, radiological and pathological features in age-matched Ashkenazi Jewish (AJ) women with breast cancer with and without BRCA1 and BRCA2 mutations. Am Soc Clin Oncol A1649, 2001.

k) Others

Cervical cancer screening:

Foulkes W: The health of the nation. *Br. Med. J.* **303**: 311-312, 1991.

Conference report:

Foulkes W: Genetics of Cancer. *Lancet* **340**: 1402-1403, 1992.

Castration and Sex:

Foulkes W: Male and female castration. *Br. Med. J.* **308**:416, 1994.

Authorship criteria:

Foulkes WD and Neylon N: Redefining authorship. *Br. Med. J.* **312**: 1423, 1996.

Book review:

Foulkes W. *Hidden Histories of Science* Edited by Robert B Silvers. New York Review Books. *Br. Med. J.* **311**:1101, 1995.

JOURNAL REFEREE

***Ad hoc*-more than 10 reviews**
Journal of Medical Genetics
International Journal of Cancer
British Journal of Cancer

Between 5 and 10 reviews
Nature Genetics
Lancet
Journal of the National Cancer Institute
Cancer Research
Clinical Cancer Research
European Journal of Cancer

Between 1 and 5 reviews
American Journal of Human Genetics
Lancet Oncology
Human Mutation
Oncogene
Gastroenterology
Prostate
Journal of Clinical Pathology
Oncology Research
Molecular and Cellular Probes
Canadian Journal of Oncology

BOOK REFEREE

Cambridge University Press

THESIS DEFENCE/REFEREE/COMMITTEE

PhD, McGill Dept. Biology
Ronald Lafreniere, June 17, 1997.

Ph.D, McGill Dept Biology
Adriana Diaz Anzaluda,
April 1999-current

PhD, McGill Dept Oncology
David Hamilton
May 2000-current

MSc, McGill Dept. Epidemiology and Statistics
Hela Makni, April 2000

MSc, McGill Dept. Biology
Sahar Sibani, January 2001

MSc, McGill Dept. Human Genetics
Andrea Karin Lawrance
May 2001-current

INVITED TALKS and SEMINARS, 1995-2006

Scientific Audience

a) International

September 19, 1995
Title:*Increased risk of squamous cell carcinoma of the head and neck in association with a family history of this cancer*.
Vermont Cancer Center
Burlington, Vermont

May 9, 1996
Title: *Genetics of head and neck cancer*
Epidemiology of head and neck cancer meeting
IARC, Lyon, France

December 9, 1996
Title: *Resolving uncertainty in hereditary breast and ovarian cancer*
Beatson Institute for Cancer Research
Glasgow, Scotland

December 10, 1996
Title: *Developing a Cancer Genetics Service*
Department of Medical Genetics, University of Glasgow,
Yorkhill Hospital
Glasgow, Scotland

February 13, 1998
Title: *Epidemiological and clinical studies of cancer genetics in the Ashkenazi Jewish population.*
Department of Human Genetics Seminar
Schwartz Building, Memorial Sloan-Kettering Cancer Center,
New York, New York.

March 12, 1998
Title: *The genetics of breast cancer in the Ashkenazi Jewish population*
Department of Epidemiology,
MD Anderson Cancer Center,
Houston, Texas.

March 12, 1998
Title: *Familial multinodular goitre and hereditary non-medullary thyroid cancer*
Department of Endocrinology,
MD Anderson Cancer Center,
Houston, Texas.

May 1, 1998
Title: *Endocrine cancers*
Department of Human Genetics
A "genetics of human cancer" course lecture
Memorial Sloan-Kettering Cancer Center,
New York, New York.

September 15, 1998
Title: *Overview of studies of prognosis in familial and hereditary breast cancer*
Breast Cancer Linkage Consortium,
Dublin, Ireland.

September 17, 1998
Title: *Genetics of Breast Cancer*
Division of Investigative Sciences,
Imperial College of Science and Medicine,
Hammersmith Hospital, London

September 18, 1998
Title: *Cancer Genetics: Much Ado about Nothing?*
GlaxoWellcome Medicines Research Centre,
Gunnels Wood Lane
Stevenage, Herts, UK.

October 5, 1998
Title: *Research in progress*
Vermont Cancer Center retreat,
Baldwin's Creek,
Bristol,VT, USA

May 26, 1999
Title: *The influence of familial and hereditary factors on the clinicopathological features and prognosis of breast cancer*
Department of Epidemiology
Fred Hutchinson Cancer Research Center
Seattle, WA, USA

September 2, 1999
Title: *Recent advances in cancer genetics*
Division of Genetic Medicine, Department of Medicine,
Vanderbilt University
Nashville, TN, USA.

December 3, 1999
Title: C*linicopathological features and prognosis of hereditary breast cancer*
Netherlands Cancer Institute

Antoni van Leeuwenhoek Huis,
Amsterdam, Netherlands

December 16, 1999
Title: C*linical, Pathological and Survival studies in hereditary breast cancer*
Duke University Medical Center
Duke University
North Carolina, USA

September 25, 2000
Title: Hereditary breast cancer: genes, risks and outcome
University of Newcastle Medical School
University of Newcastle,
Northumberland, UK

November 28, 2001
Title: Clinicopathological studies of hereditary breast cancer
CHUV,
University of Lausanne
Lausanne, Switzerland

March 27, 2003
Title: Founder populations and cancer genetics: a view from just north of here
NHGRI, Division of Intramural Research Seminar Series,
NIH,
Bethesda, MD, USA

September 22, 2003
Title: *Clinico-pathological features of BRCA1-related breast cancer*
Division of Medical and Molecular Genetics
Guy's Hospital,
London, UK

September 23, 2003
Title: *Five things I learnt about BRCA1-related breast cancer in the last year*
Gjesteforelesning,
Gades Institute,
Haukeland University Hospital
Bergen, Norway

September 24, 2003
Title: *Five things I learnt about BRCA1-related breast cancer in the last year*
Netherlands Cancer Institute
Amsterdam, Netherlands

February 2, 2006
Title: *Genetic Risk Assessment*

5th International "From Gene to Cure" Congress
Vrije Universiteit Amsterdam
Amsterdam, Netherlands

February 2, 2006
Title: *Prevention of Hereditary Breast Cancer*
5th International "From Gene to Cure" Congress
Vrije Universiteit Amsterdam
Amsterdam, Netherlands

April 20, 2006
Title: *Clinico-pathological features of basal-like/BRCA1 tumors*
"Basal-like and BRCA1-associated Breast Cancer" meeting
Harvard Club
Boston, MA, USA

August 16, 2006
Title: *Recent advances in understanding of the inherited susceptibility to cancers of the prostate, pancreas, stomach and colorectum*
Australian Ovarian Cancer Study and the Family Cancer Clinics of Australia
Couran Cove Island Resort
Stradbroke Island, Australia

August 18, 2006
Title: *Hereditary breast cancer: from pathology to treatment and beyond*
Australian Ovarian Cancer Study and the Family Cancer Clinics of Australia
Couran Cove Island Resort
Stradbroke Island, Australia

October 28, 2006
Title: Breakthrough treatments for BRCA1 and BRCA2 mutation carriers
10th Annual Cincinnati Comprehensive Breast Cancer Conference
Cutting Edge Strategies in Breast Cancer: The next decade
Cincinnati, OH, USA

November 9, 2006
Title: Hereditary breast cancer: from pathology to treatment and beyond
Cancer Colloquia IV: Cell and Molecular Biology of Breast Cancer
University of St-Andrews
St-Andrews, Scotland

b) National

April 26, 1996
Title: *Genetics of head and neck cancer*
Cancer Genetic Epidemiology Workshop
Environmental Health Centre

Ottawa, Ontario

February 24, 1999
Title: *Genetics of Breast Cancer: some observations from the study of founder populations in Quebec*
Division of Cancer Biology Research Seminar,
Sunnybrook and Women's College Hospital Health Sciences Centre,
Toronto, ON, Canada

May 19, 1999
Title: *Genetics of Breast and Ovarian Cancer*
"New Developments in prenatal diagnosis and medical genetics"
University of Toronto CME course
Toronto, ON, Canada

June 20, 1999
Title: *Node negative breast cancer in Ashkenazi Jewish women has a very good prognosis if the tumor is both HER2 and BRCA1 germ-line mutation negative*
Reasons for Hope: NCIC/CBCRI conference
Toronto, ON, Canada

June 21, 2001
Title: *Treatment issues in hereditary breast cancer*
Theme: The genetic basis of disease
Canadian Federation of Biological Societies, 44th annual meeting.
Ottawa Congress Centre
Ottawa, ON, Canada

October 7, 2004
Title: *Exons, Introns, Enhancers, Deletions and Founders: an overview of HNPCC in Quebec*
Oncogenetics: Achievements and Challenges,
17ieme entretiens du Centre Jacques Cartier
Crowne Plaza Hotel, Montreal, Quebec

June 15, 2006
Title: *Genetics and Breast Cancer: An update*
Toronto Breast Cancer Symposium 2006
Metro Toronto Convention Center, Toronto, Ontario

c) Local/Provincial

September 28, 1995
Title: *Familial Risks of Squamous Cell Carcinoma of the Head and Neck*
Annual Meeting of Quebec ORL Society
Montibello, Quebec

March 26, 1996
Title: *p16 and Familial Cancer*
Institut de Cancer de Montreal - Hopital Notre Dame
Montreal, Quebec

June 19, 1996
Title: *A p16 mutation in a family with multiple cancers*
Les Journées de Génétique Humaine - Réseau de Médicine Génétique Appliqués du FRSQ
Montreal, Quebec

October 24, 1997
Title: *A gene for familial multinodular goitre maps to chromosome 14q*
Annual Congress of the Quebec ORL Association,
Chateau Frontenac,
Quebec City, Quebec

November 20, 1997
Title: *The genetics of breast cancer*
Annual Scientific Meeting of Clinical Biochemists of Quebec,
Hotel Vogue,
Montreal, Quebec

May 29, 1999
Title: *Hereditary predisposition to breast and ovarian cancer*
Annual Congress of the Quebec Obstetrics and Gynecology Association (AOGQ)
Hotel Delta Sherbrooke,
Sherbrooke, Quebec

September 20, 2002
Title: *Genetic testing for colorectal cancer*
3rd Annual Montreal Colon and GI cancers conference
Queen Elizabeth Hotel
Montreal, Quebec

October 24, 2002
Title: *Genetics of Skin Cancer*
247th Scientific meeting of the Montreal Dermatological Society
Royal Victoria Hospital
Montreal, Quebec

October 4, 2002
Title: *Screening or Risk Reduction?*
1st International Cancer Prevention Symposium-Chagnon Foundation
Ritz Carlton Hotel,
Montreal, Quebec

November 13, 2002

Title: *Genetics of Breast Cancer*
Cité de la Santé Hematology/Oncology Group
Laval, Quebec

June 25, 2004
Title: *Screening of High Risk Patients*
23rd International Congress of Radiology of the International Society of Radiology
Palais des Congrès, Montreal, Quebec

October 7, 2004
Title: Exons, *Introns, Enhancers, Deletions and Founders: an overview of HNPCC in Quebec*
Oncogenetics: Achievements and Challenges,
17ieme entretiens du Centre Jacques Cartier
Crowne Plaza Hotel, Montreal, Quebec

November 25, 2004
Title: *Survol sur la génétique et la prise en charge du cancer colorectal héréditaire*
Centre intégré de lutte contre le cancer de la Montérégie, Réseau Cancer Montérégie
Hôtel Gouverneur Île Charron, 2405 Île Charron,
Longueuil, Quebec

December 15, 2004
Title : Genetic diseases in the adult: New opportunities
Hôtel Vogue, Montreal, Quebec

September 28, 2005
Title: Genetics of colorectal cancer: What's new?
CCMG 2005 Annual Meeting
Château Bromont
Bromont, Quebec

October 12, 2005
Title: Genetic influence of breast and gynecological cancers in pre-menopausal women
10th McGill International Symposium on Reproductive Endocrinology & Infertility and Women's Health
Centre Mont-Royal, Montreal, Quebec

October 20, 2005
Title: Overview - 10 years of BRCA1 and BRCA2
BRCA: Today & Tomorrow
First International Symposium on the Hereditary Breast and Ovarian Cancer Susceptibility Genes
Marriott Château Champlain, Montreal, Quebec

October 20, 2005
Title: Outcome following BRCA1/2 related breast cancer

BRCA: Today & Tomorrow
First International Symposium on the Hereditary Breast and Ovarian Cancer Susceptibility Genes
Marriott Château Champlain, Montreal, Quebec

d) Institutional

January 11, 1996
Title: *Breast Cancer Syndromes*
Endocrinology Research Seminar - Royal Victoria Hospital
Montreal, Quebec

February 28, 1996
Title: *Familial Breast Cancer*
Oncology Rounds - Royal Victoria Hospital
Montreal, Quebec

February 29, 1996
Title: *Preventive Surgery and the High-risk Patient*
Surgical Grand Rounds - Royal Victoria Hospital
Montreal , Quebec

March 29, 1996
Title: *Germline mutations in p16 and the risk of cancer*
McGill Genetics rounds: Case presentations-Royal Victoria Hospital
Montreal, Quebec

November 8, 1996
Title: *Controversies Surrounding New Genetic Testing* (Panel Discussion)
47th McGill University Annual Refresher Course for Family Physicians
Montreal, Quebec

November 14, 1996
Title: *The role of Preventive Surgery in the High-risk Individual*
Surgical Grand Rounds - Sir M.B. Davis Jewish General Hospital
Montreal , Quebec

November 22, 1996
Title: *Resolving uncertainty in hereditary breast and ovarian cancer*
McGill Genetics Rounds - Montreal Children's Hospital
Montreal, Quebec

November 25, 1996
Title: *Familial Cancer* (with Dr. Patricia Tonin)
Grand Medical Rounds - Sir M.B. Davis Jewish General Hospital
Montreal, Quebec

February 20, 1997
Title: *Genetics and epidemiology of non-medullary thyroid cancer*
Endocrinology rounds,
Montreal General Hospital
Montreal, Quebec.

March 20, 1997
Title: *Methods and recent results in the genetics of cancer susceptibility*
Montreal Cancer Research Group,
McGill Cancer Centre,
Montreal, Quebec.

November 13, 1997
Title: *The genetics of breast cancer*
Department of Epidemiology and Biostatistics,
Fall Seminar Series,
McGill University, Montreal

November 24, 1997
Title: *Female cancer and genetics*
Department of Obstetrics and Gynaecology Grand Rounds
Primrose Amphitheatre, Royal Victoria Hospital,
Montreal, Quebec.

December 5, 1997
Title: *Female cancers and genetics*
Department of Obstetrics and Gynaecology Grand Rounds
Block Amphitheatre, SMBD-Jewish General Hospital,
Montreal, Quebec.

December 10, 1997
Title: *Recent advances in cancer genetics*
Department of Medicine Grand Rounds
JSL Browne Amphitheatre, Royal Victoria Hospital,
Montreal, Quebec.

December 15, 1997
Title: *Breast cancer: endocrine and genetic factors* (with Professors M. Pollak and L. Pinsky)
Department of Medicine Grand Rounds
Block Amphitheatre, SMBD-Jewish General Hospital,
Montreal, Quebec.

February 5, 1998
Title: *Genetics of breast and colorectal cancer*
Department of Surgery Grand Rounds
Osler Amphitheatre, Montreal General Hospital,

Montreal, Quebec.

February 17, 1998
Title: *Cancer genetics: an introduction*
Department of Medicine Grand Rounds
Osler Amphitheatre, Montreal General Hospital,
Montreal, Quebec.

April 24, 1999
Title: *Hereditary ovarian cancer*
4th McGill International Symposium on reproductive endocrinology and infertility
Jeanne Timmins Amphitheatre,
McGill University, Montreal.

May 12, 1999
Title: *Recent advances in breast and ovarian cancer genetics*
Surgical Grand Rounds,
Royal Victoria Hospital,
McGill University, Montreal

August 25, 1999
Title: *The role of BRCA1 and BRCA2 in breast and ovarian cancer*
Obstetrics and Gynecology Rounds
Royal Victoria Hospital,
McGill University, Montreal

December 13, 1999
Title: *Genetics and Adult Onset diseases: A changing role for medical genetics*. (with Prof. D. Rosenblatt)
Medical Grand Rounds,
Sir M.B. Davis-Jewish General Hospital,
McGill University, Montreal

January 12, 2000
Title: *Genetic predisposition and outcome from cancer*
Montreal Cancer Research Group,
McGill Cancer Centre,
Montreal, Quebec.

February 24, 2000
Title: *Non-medullary thyroid cancer*
Endocrinology Grand Rounds
Sir MB Davis-Jewish General Hospital
Montreal, Quebec.

March 15, 2000 (with Ms. Lidia Kasprzak and Dr. Georges Chong)
Title: *Genetics and Cancer: How mutation analysis affects clinical management*

Medical Grand Rounds
Royal Victoria Hospital
MUHC, Montreal, Quebec

April 4, 2000 (with Ms. Lidia Kasprzak)
Title: *Colorectal Cancer Genetics: How mutation analysis affects clinical management*
Medical Grand Rounds
Montreal General Hospital
MUHC, Montreal, Quebec

November 7, 2000
Title: *Management of Hereditary Breast and Ovarian Cancer*
Medical Grand Rounds
Montreal General Hospital
MUHC, Montreal, Quebec

November 8, 2000
Title: *Management of Hereditary Breast and Ovarian Cancer*
Medical Grand Rounds
Royal Victoria Hospital
MUHC, Montreal, Quebec

October 22, 2001
Title: *Management of Hereditary Breast and Ovarian Cancer: Prevention, Early Detection and Treatment*
Medical Grand Rounds
Sir M.B. Davis-Jewish General Hospital,
McGill University, Montreal

November 17, 2001
Title: *McGill Program in Cancer Genetics: Bringing together human genetics and oncology*
McGill Oncology Research Retreat,
November 16-17,
Hotel Days Inn,
Montreal

December 16, 2002
Title: *Genetics of Cancer: an update*
MUHC Radiation Oncology Group
Montreal General Hospital

November 23, 2004
Title: *Clinicopathological features of Hereditary Breast Cancer: Ten years on*
MUHC Clinical and Research Seminar
Meakins Auditorium
McIntyre Building
McGill University

December 15, 2004
Title: *Genetics of Colorectal cancer*
GI residents
Montreal General Hospital

Lectures to Interested Groups and/or the General Public

October 26, 1996
Title: *Risk factors, prevention and early diagnosis in prostate cancer*
First Patient Advocates for Advanced Cancer Treatment (PAACT) Prostate Cancer Conference,
Grand Rapids, MI, USA

May 22, 2001
Title: *Genetics and Cancer: Prevention, Early Diagnosis and Treatment*
Research Governor's Society First Lecture Series
Lady Davis Institute for Medical Research,
Montreal, Quebec.

October 24, 2001
Title: *Genetic Testing for Cancer Susceptibility*
38th Annual André Aisenstadt Clinical Day
The Use of Genetic tests in Medical Diagnosis and Treatment
Sir M.B. Davis-Jewish General Hospital,
McGill University, Montreal

September 18, 2002
Title: *Genetic testing for colorectal cancer*
3rd Annual Montreal Colon and GI cancers pre-conference lay workshop
Queen Elizabeth Hotel
Montreal, Quebec

October 1, 2002
Title: *Genetics of Breast Cancer*
CanSupport Public Lecture
Omni Hotel,
Montreal, Quebec

September 27, 2004
Title: *The Why, Where and How of genes and diseases in the Jewish population*
National Council of Jewish Women of Canada
The power of genealogy
Gelber Conference Center Montreal, Quebec

September 19, 2005
Title: *Role of genetic factors in cancer & familial diseases*

National Council of Jewish Women of Canada
Gelber Conference Centre
Montreal, Quebec

Sabine Mai, Ph.D. CURRICULUM VITAE

*

331 Beaverbrook Street
Winnipeg MB R3N 1M9
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*

Manitoba Institute of Cell Biology
675 McDermot Avenue
Winnipeg MB R3E 0V9
Phone: 204-787-2135
FAX: 204-787-2190
e-mail: **smai@cc.umanitoba.ca**
websites: **http://www.umanitoba.ca/institutes/manitoba_institute_cell_biology/**
http://www.umanitoba.ca/institutes/manitoba_institute_cell_biology/GCCRD/Index3.htm
http://www.itmhrt.ca

*

EDUCATION

Bachelors	University of Cologne (Germany)	Biology	1981
Masters	University of Cologne (Germany)	Biology	1985
Masters	University of Cologne (Germany)	French	1985
Masters	University of Cologne (Germany)	Pedagogic	1985
Ph.D.	University of Karlsruhe (Germany)	Mol. Biology	1991

RESEARCH TRAINING during Ph.D.

DATES	PLACE	HOST(S)
1986-1988:	Tel-Aviv University, Tel-Aviv, Israel	Drs. Y. Koltin, S. Lavi, E. Waldstein
1990:	Imperial Cancer Research Fund (ICRF), London, U.K.	Dr. H. Land

RESEARCH TRAINING after Ph.D.

1991:	Turku University, Finland
1991-1995	Basel Institute for Immunology, Switzerland
1993/1994:	National Institutes of Health

(NIH/NCI), USA

EMPLOYMENT HISTORY

DATES	PLACE	TITLE
1991-1995	Basel Institute for Immunology, Switzerland	Member
1995-present	Manitoba Institute of Cell Biology	Senior Investigator
1995-2000	Department of Physiology	Assistant Professor
4/98-2001	Department of Human Genetics	Assistant Professor
5/99-present	The Genomic Centre for Cancer Research and Diagnosis (MICB)	Director
3/00-03/05	Department of Physiology	Associate Professor
3/00-03/05	Dept. of Biochemistry and Medical Genetics	Associate Professor
4/05-present	Department of Physiology	Professor
4/05-present	Dept. of Biochemistry and Medical Genetics	Professor

INTERNATIONAL RESEARCH ACTIVITES

DATES	PLACE	TITLE
4/00-5/00	Dept of Pharmacology, Chiang Mai University	Visiting Prof; Lecturer
12/00	Karolinska Institute, Stockholm, Sweden	Visiting Scientist
12/01-02/02	German Cancer Research Center, Heidelberg	Visiting Scientist
11/03-12/03	Karolinska Institute, Stockholm, Sweden	Visiting Scientist

AWARDS

Bafög stipend	1978-1985
Bafög Auslandsstipendium	1980-1981
Deutscher Akademischer Auslandsdienst (DAAD)	1986, 1987
NCIC Award for New Investigators	1995
Braidwood Jackson Memorial Award	1995
Dr. Saul Highman Memorial Award	1999
Rh Award (Basic Science)	2000
MMSF Basic Science Career Development Award	2000-2003
University of Manitoba Outreach Award	2001
US Army Breast Cancer Concept Award (shared with Drs. William Foulkes and Yuval Garini)	2004
University of Manitoba Merit Award (Research and Teaching)	2005

PROFESSIONAL SOCIETIES

American Association for Cancer Research

PROFESSIONAL ACTIVITIES

1) External grant reviewer for NSERC, MRC, MHRC, CIHR, Valorisation Recherche Québec, NCIC, MMSF
2) External referee for Proc. Natl. Acad. Sci (USA), Oncogene, J. Cell Physiol., Gene, Mol. Cell. Biol., Experimental Cell Research, Genes, Chromosomes and Cancer
3) Manitoba Institute of Cell Biology, Recruitment Committee, 1996-present
4) Department of Immunology, Recruitment Committee, 1999-present
5) Manitoba Institute of Cell Biology Animal Care Committee, 1997-present
6) Reviewer of MRC National Graduate Students Research Poster Competition, 1999
7) Reviewer of Manitoba Student Poster Competition, Research Day, 2000, 2001.
8) Genomic Centre for Cancer Research and Diagnosis: User Committee: 2001-present
9) CIHR Strategic Training Program "Innovative Technologies in Multidisciplinary Health Research training" selection committee: 2002-present
10) CIHR Strategic Training Program "Innovative Technologies in Multidisciplinary Health Research training" Annual Review Committee: 2003-present
11) CIHR Fellowship – Post- Ph.D. (FPF) committee: 2002-2006
12) Member of the University of Manitoba Senate: 2003-2006
13) Panel J Member NCIC: 2005-

PATENTS:

1) Mai, S. "Method and marker for the identification of pre-malignancy and malignancy and their therapeutic intervention". – Serial No. 09489288.
2) Mai, S., Chuang, T., Moshir, S, Garini, Y. Method of monitoring genomic instability by 3D microscopy and analysis. – Serial No. 9157-51.
3) Garini, Y, Darai, E. and Mai, S. Method and apparatus for genomic analysis.
4) Mai, S. Garini, Y, Sarkar R, Vermolen BJ. Methods of detecting and monitoring cancer using 3D analysis of centromeres. # 15502-6

RESEARCH OVERVIEW

Genomic instability is a hallmark of pre-neoplastic and neoplastic cells. The mechanisms underlying the induction of genomic instability in cells have been poorly elucidated.

My laboratory was first to demonstrate that c-Myc deregulation induces locus-specific genomic instability (Mai, 1994) and karyotypic instability (Mai *et al.*, 1996a). This finding was later confirmed by Felsher and Bishop (1999). Using c-Myc-inducible cell lines, we have identified the following genes as targets of c-Myc in genomic instability: *dihydrofolate reductase* (*DHFR*) (Mai, 1994, 1996b, Taylor *et al.*, 1997, Taylor and Mai, 1998), *ribonucleotide reductase R2* (Kuschak *et al.*, 1999a), *cyclin D2* (*CCND2*) (Mai *et al.*, 1999), and *ornithine decarboxylase* (*ODC*) (Smith *et al.*, 2002, 2003). For review, see Mai and Mushinski, 2003.

Our ongoing work focuses on the initiating mechanisms of c-Myc-mediated genomic instability. This work is important for our understanding of oncogenesis. Furthermore, we apply our knowledge on genomic changes in cancer cells to the analysis of patient samples. Using advanced technologies, we can detect cancer cells earlier than standard procedures. We will apply this knowledge to patient samples with the goal to improve early detection, surveillance and monitoring.

Details on basic research and translational research are given in the two following sections.

Basic research focus.

1) ***c-Myc-induced illegitimate DNA replication and re-organization of the genome.***

We have shown that c-Myc deregulation induces illegitimate replication of the mouse *ribonucleotide reductase R2* (*R2*) gene (Kuschak *et al.*, 2002). This finding is novel in two ways, i) it shows that replication initiation does not have to occur in fixed regions of the mammalian genome. Rather, replication may occur at different sites. ii) c-Myc can act as a licensing factor for DNA replication. In addition, under conditions of induced c-Myc deregulation, we have observed genome-wide alterations in replication patterns.

To further investigate this process of Myc-dependent DNA replication, we propose to investigate the universal role of c-Myc in DNA replication through i) a genome-wide screen of c-Myc-dependent replication initiation. Our preliminary work has indicated that c-Myc-deregulation affects overall replication patterns throughout the mouse genome. ii) We will examine previously identified c-Myc target genes in genomic instability. We will examine whether or not their replication is altered under conditions of c-Myc deregulation. iii) We propose to study the involvement of c-Myc in the replication machinery at the *R2* and other gene(s).

2) ***c-Myc induces genomic instability and apoptosis; mutant Myc proteins, however, have lost this ability and induce genomic instability in the absence of apoptosis***. We have demonstrated that wild-type (wt) c-Myc is able to promote both genomic instability and apoptosis, while mutant (mut) c-Myc proteins, that are common in Burkitt lymphoma patients, do not stimulate apoptosis, but merely promote genomic instability (Fest *et al.*, 2002). Non-random genomic aberrations are caused by the deregulated expression of wild-type *vs.* myc box II-deleted D106 proteins (Fest *et al.*, 2005). The non-random genomic alterations differ between wt and mut Myc overexpressing cells (Fest *et al.*, 2005). The tumorigenic potential of the cells expressing wt *vs.* mutant c-Myc was examined in SCID mice: Only wt-Myc overexpressing cells conferred tumorigenic potential, while D106 protein was unable to initiate tumor formation in SCID mice.

3) ***c-Myc induces the formation of extrachromosomal elements (EEs).*** Work carried out in my laboratory has demonstrated for the first time the induction of extrachromosomal elements (EEs) as a result of c-Myc deregulation. This work allowed us to identify some of the genes that are present on EEs (Mai *et al.*, 1996a,b, Mai *et al.*, 1999, Kuschak *et al.*, 1999, Smith *et al.*, 2003). We are now

analyzing the formation, structure, and function of these EEs. EEs isolated from MycER^{TM}-activated cells are significantly larger than EEs isolated from non-MycER^{TM}-activated control cells. c-Myc-induced EEs are associated with proteins. Immunohistochemistry and western blot analyses using pan-histone-, H3 phosphorylation-, and H4 acetylation-specific antibodies indicate that the majority of EEs isolated from MycER^{TM}-activated cells carry transcriptionally active chromatin. In addition, we were able to show that the c-Myc-activated EEs can replicate automomously (Smith *et al*., 2003). While some of the genes on c-Myc-activated EEs have been identified in my laboratory, we wish to assess, in a genome- wide fashion, which other genes are present on these EEs. To this end, we will examine the use of EEs as probes for microarrays.

4) ***Plasmacytoma development in p53-deficient BALB/c mice.*** The induced expression of c-Myc in plasmacytomas (PCTs) in BALB/c mice is regularly associated with non-random chromosomal translocations that juxtapose the *c-myc* gene to one of the immunoglobulin (*Ig*) loci on chromosome 12 (*IgH*), 6(*IgK*) or 16(*IgL*). The most characteristic feature of the PCT-associated chromosomal translocation is that the ratio of the typical [T(12;15)] versus the variant translocation [T(6;15)], which differs significantly depending on the PCT-induction methods. More than 90% of pristane (2,6,10,14-tetramethyl-pentadecane)-induced PCTs carry the typical T(12;15) translocation whereas the percentage of the variant T(6;15) is less than 10% (Wiener *et al.*, 1980; Ohno *et al.*, 1984a; Potter, 1997).

We have described the generation of PCTs in p53-deficient BALB/c mice. Interestingly, p53-/- mice develop PCTs earlier than their wild-type p53+/+ littermates. p53-/- mice also show an increase in variant translocations involving c-*myc* and *immunoglobulin* genes (Mai and Wiener, 2002). PCTs that carry variant T(6;15) translocations become as frequent as those with typical T(12;15) translocations (41.66%). In addition, in the absence of p53, the number of translocation-negative PCTs increases from less than 1% to 16.66%. The important issues that need to be answered deal with the generation and survival of the *c-myc/Ig*-carrying B cells. We will address the question whether or not both types of translocations (typical and variant translocation) occur at equal ratios. Usually the typical translocation (T12;15)- carrying B cells would survive, while the variant translocation (T6;15, T15;16) -carrying B cells would be eliminate d by p53-dependent apoptosis. However, under conditions of p53 deficiency, both types of translocation-carrying cells would survive at equal ratios.

5) ***The three-dimensional organization of the mammalian nucleus.*** Using high resolution deconvolution microscopy, we have elucidated the three-dimensional (3D) organization of telomeres and chromosomes in the interphase nuclei of normal, immortalized and tumor cells. Here, we have established that the mammalian telomeres are organized dynamically and non-randomly in the 3D nucleus of normal cells around a central telomeric disk. The telomeric disk forms in late G2. On the other hand, 3D nuclei from tumor cells display a disrupted 3D nuclear telomeric organization: telomeres in tumor cell nuclei form aggregates of various numbers and sizes (Chuang *et al*., 2004). Our ongoing studies focus the mechanism of telomere aggregation and their link to the initiation and/or promotion of genomic instability.

6) ***c-Myc alters the 3D organization of the nucleus and thereby causes chromosomal rearrangements.*** We have recently shown that c-Myc deregulation induces alterations in the 3D organization of telomeres. Telomeres form aggregates of various numbers and sizes. Some of these aggregates represent telomeric fusions. Chromosomes whose ends are fused telomerically often form dicentric chromosomes. These chromosomes break in the subsequent anaphase and generate unbalanced translocations and terminal deletions. The initiation of such breakage-bridge-fusion cycles continues until no more free chromosome ends persist (Louis *et al*., 2005; Mai and Garini, 2005, Mai and Garini, 2006).

Translational research focus.

Basic research into cancer cell development can be used to examine the nature of cells, *i.e*. to determine whether cells are normal or tumor cells. It is my vision to translate the knowledge gained in my laboratory into clinical applications. This work is ongoing, in collaboration with clinical colleagues, using various cancer models and has led to the filing of three patent applications. Using advanced technologies (such as fluorescent *in situ* hybridization, spectral imaging, three-dimensional imaging and analysis), we identify genomic instability early. This will allow for the early detection of cancer and will also impact on the surveillance of the patient and allow for criteria to assess treatment success.

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48) November 2000. “c-Myc-mediated locus-specific and karyotypic instability”. NIH/Bethesda, Maryland, USA.

49) December 2000. “Translocation-negative mouse plasmacytomas as a model for translocation-negative human Burkitt-like lymphomas” AACR special conference. La Jolla, USA.

50) March 2001. “Transient excess of wt Myc activity in BAF3 cells can elicit genomic instability, proliferation and apoptosis.” AACR, New Orleans (Fest, T., Mai, S.).

51) March 2001. “c-Myc Inducible Chromosomal and Extrachromosomal Amplification of R2 and ODC. AACR, New Orleans (Smith, G.M, Dushnicky, L., Symons, S., Wright, J.A., and Mai, S.)

52) March 2001. “c-Myc-Induced Gene Amplification Over-rides Replication Control.” AACR, New Orleans (T. I. Kuschak, F. Wiener, B. C. Kuschak, C. Taylor, J. A. Wright, S. Mai).

53) April 2001. “Plasmacytoma development in p53-deficient BALB.cRb6.15 mice”. Symposium on Plasma Cell Tumor Formation. NIH/NCI.

54) May 2001. D and E cyclin deregulation in chronic lymphocytic leukemia (CLL). (Paul, J.T., Johnston, J.B., Mai, S.). IX IWCLL. Abingdon, Oxfordshire, UK.

55) June 2001. “c-Myc induced *dihydrofolate reductase* (*DHFR*) gene amplification as genomic biomarker in cervical neoplasia.” The Society of obstetricians and gynaecologists of Canada. (Guijon, F, Mai, S., Paraskevas, M.).

56) June 2001. “c-Myc-Induced Gene Amplification Over-rides Replication Control.” AACR, New Orleans (T. I. Kuschak, F. Wiener, B. C. Kuschak, C. Taylor, J. F. Mushinski, J. A. Wright, S. Mai). Oncogene Meeting. Frederick, MD, USA.

57) October 2001. “New aspects of c-Myc-dependent genomic instability.” Special AACR meeting. Palm Desert. USA.

58) October 2001. c-MYC overexpression in Ba/F3 cells simultaneously elicits genomic instability and apoptosis. Hematology/Oncology Meeting. France.

59) March 2002. Les nouveaux aspects de l’instabilite genomique induite par c-myc. Fest, T., Mougey, V., Silva, S., Smith, GM, Kuschak, TI, Kuttler, F, Mai, S. Congres de la societe française d’hematologie. Paris.

60) July 2002. Development of Imaging Tools for Combined Protein – FISH Analysis (CPFA). Sabine Mai, Petra Boukamp, Jörg G. G. Bode. Euroconference on Quantitative Molecular Cytogenetics. Stockholm. www.isbe.man.ac.uk/QMC/

61) November 2002. "Novel aspects of c-Myc-dependent genomic instability". OCI. Toronto.

62) February 2003. “c-Myc-induced genomic instability”. Karolinska Institute, Stockholm, Sweden.

63) February 2003. “Genomic instability is defined by the 3D intranuclear space”. Karolinska Institute, Stockholm, Sweden.

64) March 2003. “Novel aspects of c-Myc-induced genomic instability”. Rennes, France.

65) March 2003. “Genomic instability as a function of the 3D intranuclear space. Example: mouse plasmacytoma.” B Cell Neoplasia Meeting. Washington DC, USA.

66) May 2003. “Disruption of the three-dimensional organization of telomeres and chromosomes in tumor cells”. Canadian Genetic Disease Network. Kananaskis, Alberta.

67) June 2003. “Aberrations of the three-dimensional organization of telomeres and chromosomes in tumor cells”. FASEB conference. “Nuclear Structure and Cancer”. Vermont, USA.

68) June 2003. “Novel aspects of c-Myc-dependent genomic instability”. Symposium on hormonal carcinogenesis. Valencia, Spain.

69) November 2003. A novel 3D telomeric structure in mammalian cells is disrupted during genomic instability. International Symposium on Minimal Residual Cancer, Oslo, Norway.

70) February 2004. Genomic instability has three dimensions. National Cancer Institute, Advanced Technology Center. USA.

71) March 2004. Genomic instability disrupts three-dimensional (3D) nuclear organization of telomeres and chromosomes in mammalian cells. AACR, Orlando, USA.

72) March 2004. Genomic instability in *v-abl-myc*-induced plasmacytomas. AACR, Orlando, USA.

73) March 2004. A-Max-dependent cellular growth alterations. AACR, Orlando, USA.

74) May 2004. 4th Canadian Symposium on Telomeres and Telomerases. “Alteration of the 3D organization of telomeres and chromosomes in tumor cells and after c- Myc deregulation”. Bishop University, Sherbrooke, QC.

75) May 2004. 4th Canadian Symposium on Telomeres and Telomerases. Soumya Panigrahi, Landon Wark, Bart Vermolen, Alice Chuang, Kimberly Kotar, Linda Snell, Peter Watson, Yuval Garini, William Foulkes, Sabine Mai. “3D organization of telomeres in breast cancer cells”. Bishop University, Sherbrooke, QC.

76) May 2004. Canadian Genetic Disease Network Meeting. “Genomic instability has three dimensions”.

77) November 2004. AACR Special Conference on Telomeres and Telomerase in Cancer. San Francisco, USA. “Disorganization of th ethree-dimensional organization of telomeres in head and neck squamous cell carcinoma”. Chuang T, Chuang A, Vermolen B, Garini Y, Mai S.

78) November 2004. AACR Special Conference on Telomeres and Telomerase in Cancer. San Francisco, USA.”TeloView: a 3D analysis toolbox for measuring the organization and intensities of FISH stained telomeres in interphase nuclei”. Garini Y, Vermolen BJ, Chuang A, Chuang T, Mai S.

79) November 2004. AACR Special Conference on Telomeres and Telomerase in Cancer. San Francisco, USA. “Disruption of the three-dimensional (3D) organization of telomeres during cellular transformation”. Mai S, Gairni Y, Vermolen B, Panigrahi S, Louis SF, Guffei A, Begin LR, Chuang A, Chuang T, Hicks G, Foulkes WD.

80) November 2004. AACR Special Conference on Telomeres and Telomerase in Cancer. San Francisco, USA. “Immortalization of human lymphocytes is not sufficient to alter the 3D organization of telomeres”. Louis SF, Andersson K, Henriksson M, Klein G, Mai S.

81) November 2004. Chiang Mai University, Thailand. “The Strategic Training Program "Innovative Technologies in Multidisciplinary Health Research Training" and presentation of our new training program video (www.itmhrt.ca).

82) November 2004. Chiang Mai University, Thailand. “The three-dimensional organization of telomeres - impact on genomic instability and cancer”

83) November 2004. Mahidol University. Bangkok. Thailand.” c-Myc-dependent genomic instability and cancer”

84) November 2004. Thailand Research Fund. Bangkok, Thailand. “The Strategic Training Program "Innovative Technologies in Multidisciplinary Health Research Training" and presentation of our new training program video (www.itmhrt.ca).

85) February 2005. Microbiology and Tumorbiology Center. Karolinska Institute. Stockholm, Sweden. “c-Myc-dependent genomic instability: new insights”.

86) February 2005. University of Technology Delft. Quantitative Imaging Group. “c-Myc-induced genomic instability: pathways to cellular transformation”.

87) March 2005. Lorus Therapeutics, Toronto. The dimensional organization of the nucleus: impact for genomic instability and cancer”.

88) April 2005. AACR Anaheim, California.

Gehrke I, Garini Y, Mai S. “The three-dimensional (3D) analysis of centromeres in interphase nuclei of normal and tumor cells.”

89) April 2005. AACR Anaheim, California.

Kuttler FL, Mushinski JF, Mai S. “ c-Myc-induced genomic instability: characterization of c-Myc-induced extrachromosomal elements.”

90) April 2005. AACR Anaheim, California.

Louis SF, Garini Y, Graves A, Gruhne B, Mai S. “Mechanisms of c-Myc dependent gene amplification.”

91) April 2005. AACR Anaheim, California.

Wark LJ and Mai S. “Three-dimensional (3D) analysis of telomere positioning during the cell cycle”.

92) April 2005. University of Sherbrooke, Quebec. “The three-dimensional organization of the nucleus: role in genomic instability and cancer”.

93) April 2005. Association Cytogenetique du Quebec. Chateau Bromont., Quebec. “Conformation tridimentionelle du noyeau et emergence de l’instabilite genomique”.

94) May 2005. CIHR Strategic Training Program Meeting. Mont Tremblant. Quebec.
Gehrke I, Garini Y, Mai S. “The three-dimensional (3D) analysis of centromeres in interphase nuclei of normal and tumor cells.”

95) May 2005. CIHR Strategic Training Program Meeting. Mont Tremblant. Quebec.
Louis SF, Garini Y, Graves A, Gruhne B, Mai S. “Mechanisms of c-Myc dependent gene amplification.”

96) June 2005. FASEB Summer Conference. Vermont. USA. “c-Myc deregulation remodels the three-dimensional organization of the nucleus”.

97) June 2005. FASEB Summer Conference. Vermont. USA.
Louis SF, Garini Y, Graves A, Gruhne B, Mai S. “Mechanisms of c-Myc dependent gene amplification.”

98) June 2005. FASEB Summer Conference. Vermont. USA.
Gehrke I, Garini Y, Mai S. “The three-dimensional (3D) analysis of centromeres in interphase nuclei of normal and tumor cells.”

99) September 2005. CCMG Annual Conference. Chateau Bromont, Quebec. c-Myc remodels the three-dimensional organization of the interphase nucleus.”

100) October 20005. Cell, Molecular and Atomic Imaging Symposium. St. Boniface.
“Imaging technologies and training at the Genomic Centre for Cancer Research and Diagnosis”.

101) October 20005. Cell, Molecular and Atomic Imaging Symposium. St. Boniface.

"c-Myc deregulation remodels the 3D organization of the interphase nucleus".

102) November 2005-December 2005. "FISH and SKY: Theory and Applications". 1st international workshop in Chiang Mai, Thailand.

103-105) April 2006. AACR, Washington DC, USA.

c-Myc alters the three-dimensional order of telomeres and chromosomes in the interphase nucleus. *Sherif F. Louis, Bart Vermolen, Fabien Kuttler, Yuval Garini, Sabine Mai.* Proc Amer Assoc Cancer Res 2006;47:[4240]

The three-dimensional order of telomeres impacts on tumorigenesis. *Andrea Caporali, Landon Wark, Sabine Mai*. Proc Amer Assoc Cancer Res 2006;47:[4985]

Three dimensional organization of telomeres in hereditary and non-hereditary breast cancer. *Soumya Panigrahi, Sabine Mai, William Foulkes, Kimberley Kotar, Louis R. Bégin.* Proc Amer Assoc Cancer Res 2006;47:[4991]

106) August/September 2006. Telomeres and Genome Stability. Switzerland.

107) September 2006. Hormonal Carcinogenesis. Montpellier. France.

CURRENT SOURCES OF FUNDING

Investigator: Sabine Mai

Sponsor: CancerCare Manitoba Foundation

Project: The significance of telomeric aggregates in tumor development

Funds authorized: $50,000/annum (2006-2007)

Investigator: Peter Watson

Co-applicant: Sabine Mai

Sponsor: CIHR Research Resource Grant Award

Project: The Manitoba Tumor Bank - a critical engine for cancer research.

Funds authorized: $105,065/annum (2006-2011)

Investigator: Sabine Mai

Sponsor: The Susan G. Komen Breast Cancer Foundation

Project: The 3D telomeric signature(s) of DCIS.

Funds authorized: US $249, 805 (2006-2009)

Investigator: Sabine Mai

Sponsor: CCMF

Project: Live Cell 3D Imaging Station.

Funds authorized: $191,983 (2006)

Investigator: Sabine Mai

Sponsor: NCIC

Project: Mechanisms of chromosomal rearrangements.

Funds authorized: $450,000 (2005-2008)

Investigator: William Foulkes

Co-Investigators: Torsten Nielsen and Sabine Mai

Sponsor: CBCRA

Project: BRCA1, CDC4, cyclin E and chromosomal instability in breast cancer.

Funds authorized: $310,00 (2005-2008)

Investigator: Sabine Mai

Sponsor: NSERC

Project: Tumor progression by c-Myc

Funds authorized: $60,000 (2005-2007)

Investigator: Sabine Mai

Sponsor: CancerCare Manitoba

Project: Mechanisms of telomeric aggregate formation in mammalian interphase nuclei

Funds authorized: $48,000 (2005-2006)

Investigator: Sabine Mai

Sponsor: CIHR

Project: Mechanisms of c-Myc-induced genomic instability.

Funds authorized: $83,826/annum (2005-2008) – declined.

Investigator: Sabine Mai

Sponsor: CIHR IPM

Project: Tumor cell detection through 3D molecular imaging and analysis.

Funds authorized: $25,000 (1/2005-5/2005)

Investigator: Sabine Mai

Sponsor: CancerCare Manitoba

Project: Genomic instability in three dimensions

Funds authorized: $50,000 (2004-2005)

Investigators: William Foulkes, Sabine Mai, Yuval Garini

Sponsor: US Army Breast Cancer Concept Award

Project: Breast cancer in three dimensions

Funds authorized: $ US 80,000 (2004-2005)

Investigator: Patrick C. Choy

Co-applicant: Sabine Mai

Sponsor: Canadian Institutes of Health Research (CIHR)

Project: Maintenance Grant Application in Support of the Multi-User Flow Cytometry Laboratory in the Faculty of Medicine, U of M

Funds authorized: $ 58,352 (2004-2007)

Investigator: Jan Friedman

Co-applicant: Sabine Mai

Sponsor: Canada Foundation for Innovation (CFI)

Project: Canadian Molecular Cytogenetics Platform

Funds authorized: $ 4.5 mill. (2004-2009)

Investigator: Sabine Mai

Sponsor: Canadian Institutes of Health Research (CIHR).

Project: Strategic Training Program Grant: "Innovative Technologies in Multidisciplinary Health Research Training".

Funds authorized: 300,000.00/annum (2002-2008).

Investigator: Sabine Mai.

Sponsor: Canadian Institutes of Health Research (CIHR).

Project: "Mechanisms of genomic instability".

Funds authorized: $ 83,826 (2002-2005).

Investigator: Jim Davie

Co-applicant: Sabine Mai

Sponsor: Canada Foundation for Innovation (CFI)

Project title: "Manitoba Breast Cancer Research and Diagnosis Centre"

Funds authorized: $ 3,795 792

Investigator: Mahesh Chaturvedi

Co-applicant: Sabine Mai

Sponsor: Canada Foundation for Innovation (CFI)

Project title: "200 kV Field Emission Gun Cryo-Transmission/Scanning Electron Microscope"

Funds authorized: $ 2,994 236

Investigator: Sabine Mai

Sponsor: National Science and Engineering Research Council (NSERC).

Project: "c-Myc-dependent *DHFR* gene amplification"

Funds authorized: $ 30,000 (2001-2005)

PREVIOUS SOURCES OF FUNDING

Investigator: Sabine Mai

Sponsor: CancerCare Manitoba

Project: "Development and validation of quantitative algorithms that define the position of telomeres in the three-dimensional (3D) mammalian interphase nucleus."

Funds authorized: $50,000 (2003-2004).

Investigator: Sabine Mai

Sponsor: Paul H. T. Thorlakson

Project: "Application of Comparative Genomic Hybridization (CGH) to Genetic Analysis of Pediatric Acute Lymphoblastic Leukemia (ALL)".

Funds authorized: $24,000. (2002-2003).

Investigator: Sabine Mai

Sponsor: CancerCare Manitoba

Project: TRF2: An anaphase chromosome stabilizing protein?

Funds authorized: 49,12.00/annum (2002-2003).

Investigator: Sabine Mai

Sponsor: Canadian Institutes of Health Research (CIHR)

Project: CIHR Training Program Development Grant

Funds authorized: $ 4,000 (2001-2002)

Investigator: Sabine Mai

Sponsor: Canadian Institutes of Health Research (CIHR)

Project: “The impact of p53 on c-Myc-dependent genomic instability and neoplasia”

Funds authorized: $ 52,500,-/annum (1998-2003)

Investigator: Sabine Mai

Sponsor: Manitoba Health Research Council (MHRC) – University-Industry Collaborative Grant

Project: Early detection of cervical cancer

Funds authorized: 18,750 (2001-2002)

Investigator: Sabine Mai

Sponsor: Applied Spectral Imaging Inc.
Project: Early detection of cervical cancer
Funds authorized: 18,750 (2001-2002)

Investigator: S. Mai, J. B. Johnston

Sponsor: Canada Foundation for Innovation (CFI)

Project: "The Genomic Centre for Cancer Research and Diagnosis"

Funds authorized: $ 2.25 mill. (1999-2004)

Investigator: Sabine Mai

Sponsor: CancerCare Manitoba

Project: "Extrachromosomal c-Myc activation"

Funds authorized: $ 50,000 (2001-2002)

Investigator: S. Mai
Sponsor: National Science and Engineering Research Council (NSERC)
Project: Mechanisms of *DHFR* gene amplification upon c-Myc deregulation
Funds authorized: 29.800 (2000-2001)

Investigator: J. B. Johnston, S. Mai, A. Begleiter
Sponsor: Leukemia Research Fund of Canada
Project: "Novel mechanism of action of the nucleoside analogs in chronic lymphocytic leukemia"
Funds authorized: $ 25.000/annum (1999-2001)

Investigator: S. Mai

Sponsor: Manitoba Medical Service Foundation

Project: "Chromosome 15 rearrangements in AKR lymphomas.

Funds authorized: $ 20.000,-/annum (2000-2001)
Investigator: Sabine Mai
Sponsor: Leukemia Research Fund of Canada (LCRF)
Project: Identification of candidate genes in non-random rearrangements of chromosome 15 in AKR lymphomas with diploid or trisomic chromosome 15 constitution.
Funds authorized: 45.000 (2000-2001)

Investigator: J. B. Johnston, S. Mai, A. Begleiter
Sponsor: Manitoba Cancer Treatment and Research Foundation
Project: "Identification and functional characteristics of extrachromosomal genes in chronic lymphocytic leukemia"
Funds authorized: $50.000 /annum (1999-2000)

Investigator: S. Mai
Sponsor: Manitoba Health and Research Council (MHRC) Establishment grant
Project: "Functional interactions between c-Myc and p53 during c-Myc-dependent genomic instability and neoplasia?"
Funds authorized: year 1: $ 34.000,-; year 2: 33.000,-; year 3: 33.000,- (1997-2000)

Investigator: S. Mai
Source: National Science and Engineering Research Council (NSERC)
Project: "The DHFR gene, a target of c-Myc"
Funds authorized: $ 30.000,-/annum (1995-2000)

Investigator: J.B. Johnston, A. Begleiter, S. Mai
Sponsor: Berlex Canada Inc.
Project: A novel mechanism of action of fludarabine in chronic lymphocytic leukemia
Funds authorized: $ 56. 104 (1999).

Investigator: A. Ronald
Co-applicant: S. Mai (one of ten co-applicants)
Sponsor: Medical Research Council
Project: FACS facility
Funds authorized: $ 350.000 (1999)

Investigator: S. Mai and J. B. Johnston
Sponsor: Leukemia Research Fund of Canada
Project: "Cyclin D2 amplification in Chronic Lymphocytic Leukemia"
Funds authorized: $ 44,620/annum (1998-1999)

Investigator: S. Mai and J. Johnston

Sponsor: Manitoba Cancer Treatment and Research Foundation (MCTRF)
Project: "Genomic Instability in chronic lymphocytic leukemia"
Funds authorized: $ 49.970,85/annum (1998-1999)

Investigator: S. Mai
Sponsor: Children Hospital Research Foundation
Project: "Does N-Myc drive DHFR gene amplification in neuroblastoma?"
Funds authorized: $ 5000,-/annum (1997-1999)

Investigator: S. Mai
Sponsor: Manitoba Medical Service Foundation (MMSF)
Project: "The role of DHFR in tumorigenesis"
Funds authorized: $ 25.000,-/annum (1998-1999)

Investigator: S. Mai
Sponsor: Manitoba Health Research Council
Project: "The role of c-Myc in gene amplification"
Funds authorized: $ 48.000,- (1995-1997)

Investigator: S. Mai
Sponsor: Manitoba Cancer Foundation
Project: "The role of c-Myc in neoplasia"
Funds authorized: $ 31.500,- (1996-1997)

Investigator: S. Mai
Sponsor: Thorlakson Foundation Fund
Project: "Mechanisms of Genomic Instability"
Funds authorized: $ 15.000,- (1996-1997)

Investigator: S. Mai
Sponsor: Research Development Fund
Project: "Translocation-free juxtaposition of *c-myc* and *immunoglobulin* loci during B cell neoplasia"
Funds authorized: $ 8.000,- (1997-1998)

ADVISORY COMMITTEES:

1) Christine Vandevelde (MSc.), Department of Biochemistry, 1998-2001

2) Keding Chen (MSc.), Department of Physiology, 1997-2001

3) Theodore Kuschak (Ph.D.), Department of Microbiology, 1996-2000

4) Greg Smith (Ph.D.), Department of Biochemistry, 1998-2003.

5) Slavica Djuric-Ciganovic (MSc.), Department of Human Genetics, 1998-1999.

6) James T. Paul, (MSc.), Department of Human Genetics, 1998-2001.

7) Gracjan Bozek, Department of Physiology, 1998-2005.

8) External Ph.D. reviewer for Chen Delin's Ph.D. thesis on genomic instability and chromosomal fragmentation, The National University of Singapore. 1999.

9) External Ph.D. reviewer for the Thai Jubilee Program, Chiang Mai University. 2000.

10) External Ph.D. reviewer for Ajay Pandita, University of Toronto. 2000.

11) Kendra Caan (M.Sc./Ph.D.). Department of Biochemistry and Medical Genetics, 2000-present.

12) Co-advisor of Somchai Awakairt Ph.D., Faculty of Pharmacy, Chiang Mai, Thailand, 2000.

13) Karen Poirier, Department of Physiology, September 2000-October 2001.

14) Hortense Rabet, Department of Physiology, September 2001-May 2003.

15) Michael Lizardo, Department of Oral Biology, July 2002-May 2003.

16) Marlon Hagerty, Department of Physiology, July 2002-present.

17) Tony Chuang, Department of Physiology, July 2002-present.

18) Claire Sevenhuysen, Virology Laboratory, Winnipeg, June 2002-present.

19) Margaret Rak, Department of Chemistry, June 2003-present.

20) Sherif Louis, Department of Physiology, August 2003-present.

21) Soumya Panigrahi, Department of Physiology, January 2004-September 2006.

22) Jessica Gietz, Department of Biochemistry and Medical Genetics, January 2005-August 2005.

23) S. Maddikka. Department of Biochemistry and Medical Genetics. Candidacy committee. 2005.

STUDENTS AND POST DOCS SUPERVISED

a) M.Sc.

Paul, J.	1998-	2001	MICB, Biochem	M.Sc., 2001
Karen Poirier	2000	MSc student	Physiology/MICB	technician
Marlon Hagerty	2002	MSc. student	Physiology/MICB	MSc. in progress
Tony Chuang	2002	MSc. student	Physiology/MICB	MSc in progress

b) Ph.D.

Figueroa, R.	1995	Ph.D.	Heidelberg, Germany	Ph.D., scientist
Smith, G.	98-02	Ph.D.	MICB, Biochem	MSc, Virol. lab, Wpg
Kuschak, T.I.	96/00	Ph.D. student	MICB, Microbiol	Ph.D.02, Virol. Lab
Hortense Rabet	2001	Ph.D student	Physiology/MICB	technician
Louis, Sherif	2003	Ph.D. student	Physiology/MICB	Ph.D. in progress
Dr. Panigrahi, S	2004	Ph.D. student	Physiology/MICB	Ph.D. in progress

c) Post doctoral fellows (PDFs)

Jalava, A.	1996/7	post doc	MICB	post doc (Auburn)
Thangirala, S.	1996/7	post doc	MICB	post doc (Biochem)
Baijal, P.	1998-	Post doc	MICB	post doc in progress
Santiago Silva	2001	PDF	MICB	PDF (Sweden)
Chat Tayapiwatana	2002	PDF	MICB	PDF (Chiang Mai)
Fabien Kuttler	2004	PDF	MICB	2004-2006
Jun Li	2006	PDF	MICB	06/2006-
Hao Song	2006	PDF	MICB	start date: Oct 2006
Jana Harizanova	2006	PDF	MICB	start date: Nov 1/2006

d) Summer students/rotation students/project students

Narayansingh, R.	1996	summer student	MICB	MD. in progress
Paul, J.	1997	summer student	MICB	MSc. student (MICB)
Kwasnika, A.	1997	summer student	MICB	MSc. student
Landon Wark	2002	Summer student	MICB	Grad student/Brandon
Morgan Jarvis	2002	Summer student	MICB	Grad student/Queens
James Tsang	2002	Summer student	MICB	Grad student/U of M (U of BC)
Beaudin, J.	1997	summer student	MICB	student (U of M)
Marlon L. Hagerty	2000	summer student	MICB	2nd year student
Marc, J.	1998	student	Dept. Physiology	lecture course student
Guffei, A.	1999	summer student	MICB	high school student
Harwood, P.	1999	summer student	MICB	high school student
Vukovic, B.	1999	project student	MICB	Ph.D. student, U of T
Qing Zhao	2000	project student	MICB	student on probation
Guffei, A.	2000	summer student	MICB	student researcher
Sakar, R.	2000	summer student	MICB	high school student

Tony Chuang	2000	rotation project	MICB	resident
Marlon Hagerty	2001	Summer student	MICB	MSc student
Rahul Sarkar	2001	Summer student	MICB	informatics student
Landon Wark	2003	Summer student	MICB	Grad student/Brandon
Morgan Jarvis	2003	Summer student	MICB	Grad student/Queens
Karen Lumsden	2004	Summer student	MICB	Grad student
Landon Wark	2004	Summer student	MICB	Grad student U of M
Morgan Jarvis	2004	Summer student	MICB	Grad student/Queens
Alexander Graves	2005	Summer student	MICB	stud/Western Ontario
Jeanna Scherzcer	2005	Summer student	MICB	stud/Edinburgh
Sourabh Maiti	2005	Summer student	MICB	stud/U of M
Alexander Graves	2006	Summer student	MICB	stud/Western Ontario
Filip Misev	2006	Summer student	MICB	stud/U of Basel
Adnan Arapovic	2006	Summer student	MICB	stud/ Winnipeg

e) Visiting students and fellows supervised

a) M.Sc.

Mathilde Perrin, University of Besançon, France	2003

b) Ph.D.

Sharareh Moshir, German Cancer Research Center, Heidelberg, Germany	2002, 2003
Sibylle Ermler, German Cancer Research Center, Heidelberg, Germany	2003
Virginie Mougey, University of Besançon, France	2001
Virginie Mougey, University of Besançon, France	2002
Virginie Mougey, University of Besançon, France	2003
Andrea Caporali, University of Parma, Italy	6/8-2004
Andrea Caporali, University of Parma, Italy	6/9-2005

c) Visiting fellow

Martina Braun, EMBL Heidelberg, Germany	2000-2002

d) undergraduate students

Bettina Gruhne, University of Tuebingen, Germany	4/5-2004

f) Visiting PDFs supervised

Dr. Karin Greulich-Bode, German Cancer Research Center, Heidelberg, Germany	2002

g) CIHR trainees supervised

2002

Verayuth Praphanphoj, Dr.	Genetic Lab, Rajanukul Hospital, Thailand
Amanda Guffei	MICB
Sharareh Moshir	German Cancer Research Center
Virginie Mougey	University Hospital Jean Minjoz, France
Carolyn Gibbs	Physiology, University of Manitoba (U of M)
Steve Melnyk	Fort Richmond Collegiate
Alex Dibrov	Fort Richmond Collegiate
Shannon Neuman	MICB
Marlon Hagerty	Physiology, U of M
Tony Chuang	Physiology, U of M
Lorie Carriere	Apotex Fermentation Inc
Alicja Kellar	Apotex Fermentation Inc
Eduard Popke	Apotex Fermentation Inc
Michelle McAuley	Apotex Fermentation Inc
Margaret Rak	Chemistry, U of M
Paula Espino	MICB, Biochemistry & Med Genetics
Trung N. Le	MICB, Anatomy
Yasmin Banu	MICB
Shihua He	MICB
Lin Li	MICB, Biochemistry & Med Genetics
Virginia Spencer	MICB, Biochemistry & Med Genetics
Michaela Samek	MICB
Cheryl Taylor	MICB
Sherif Louis	MICB, Microbiology

2003

Dr. Sonia Sachot	University of Rennes1, Medicine
Dr. Mehdi Alizadeh	University of Rennes1, Medicine
Mary Ann George	Toronto
Dr. Chatchai Tayapiwatana	Chiang Mai University, Immunology
Michaela Samek	MICB
Rukmali Mendis	MICB
Dr. Tony Chuang	MICB, Physiology, U of M, HSC
Shibani Bal	Clinical Diagnostics Lab, HSC
Marlon Hagerty	MICB, Physiology, U of M
Cheryl Taylor-Kashton	MICB
Christina Walker	MICB
Margaret Rak	Chemistry, U of M
Trung N Le.	MICB, Anatomy
Lin Li	MICB, Biochemistry & Med Genetics
Sibylle Ermler	German Cancer Research Centre, Germany

Dr. Yasmin Banu	MICB
Dr. Malgorzata Burek	University of Muenster
Shannon Neumann	MICB
Paula Espino	MICB, Biochemistry & Med Genetics
Dr. Watchara Kasinrerk	Chiang Mai University, Immunology
Dr. Nusra Sittidilokratna	Mahidol University
Mike Lizardo	MICB, Oral Biology
Angela Dawson	Clinical Diagnostics Lab, HSC
Atmuri, Vasantha	U of M, Biochemistry/Med Genetics
Balakrishnan, Apoona	Fort Richmond Collegiate
Chuang, Alice	MICB
Crawford-Young, Susan	Electrical Engineering, U of M
Emberley, Ethan	U of M, Pathology
Dunn, Katherine	U of M, Biochemistry/Med Genetics
Fetterman, Christina	MICB
Law, Warren	MICB
Lee, Debbie	St George Hospital, Oncology, UK
Lewis, Anthony	U of M, Biochemistry/Med Genetics
Sourabh, Maiti	Vincent Massey Collegiate
Martelli, Lucia	U of Sao Paulo, Brazil
Morato de Oliviera, Fabio	U of Sao Paulo, Brazil
Park, Ji-Seong	MICB
Perrin, Mathilde	U of Besançon, France
Sahai, Beni	Cadham Provincial Lab
Triggs-Raine, Barabara	U of M, Biochemistry/Med Genetics
Versace, Antonietta	U of M, Biochemistry/Med Genetics
Wanichanon, Chaitip	Science Mahidol University, Thailand
Auditors:	
Bal, Shibani	HSC, Clinical Cytogenetics Lab
Sahai, Vandama	MICB
Wover, Roberta	MICB
Drobic, Bojan	MICB

2004

Piranit Kantaputra	Chiang Mai University, Thailand
Katalin Benedek	Karolinska Institute, Stockholm, Sweden
Eva Darai	Karolinska Institute, Stockholm, Sweden
Bart Vermolen	Delft University of Technology, The Netherlands
Fabio Morato de Oliveira	University of Sao Paulo, Brazil
Nutjeera Intasai	Chiang Mai University, Thailand
Vasantha Atmuri	University of Manitoba, Biochemistry/Med Genetics
Shan Li	University of Manitoba, Physiology
Anna Kania	Munster university, Germany
Silvia Rogatto	University of Sao Paulo, Brazil
Christina Fetterman	MICB

Warren Law	MICB
Zhi-Sheng Jiang	University of Manitoba, Physiology
Klaus Wroggeman	University of Manitoba, Biochemistry/Med Genetics
Ji-Seong Park	MICB
Shihua He	MICB
Roberta Wover	MICB
Tanguy Marqueste	University of Manitoba, Physiology
Gary Jones	Laboratory of Genetics, NIH, USA
Katherin Dunn	MICB
Alice Chuang	MICB

Auditors:

Fabien Kuttler	MICB
Soumya Panigrahi	MICB

2004

Betty Engracia	De La Salle University, Philippines
Nehal Patel	University of Manitoba, Winnipeg
Phatchaneeya Thammawong	Chiang Mai University, Thailand
Shauna Loewen	University of Manitoba, Winnipeg
Jinsil Kim	University of Manitoba, Winnipeg
Soumya Panigrahi	University of Manitoba, Winnipeg
Fabien Kuttler	Manitoba Institute of Cell Biology, Winnipeg
Richard Wiens	University of Manitoba, Winnipeg
Meghan Gallant	University of Manitoba, Winnipeg
Jessica Gietz	University of Manitoba, Winnipeg
Morel Rubinger	CancerCare Manitoba, Winnipeg
Mary Lynn Duckworth	University of Manitoba, Winnipeg
Dianna Martin	University of Manitoba, Winnipeg
Shahrzad Jalali	University of Manitoba, Winnipeg
Charlton Cooper	University of Manitoba, Winnipeg
Mohammad Murshedul Alam	University of Alberta, Edmonton
Juliana Cuzzi	University of Sao Paulo, Brazil
Ana Carolina Laus	University of Sao Paulo, Brazil
Andreea Nistor	University of Manitoba, Winnipeg
Tiba Tsafack	University of Yaounde I, Cameroon
M. V. Subba Reddy	University of Manitoba, Winnipeg
Saeid Ghavami	Manitoba Institute of Cell Biology, Winnipeg
Travis Clark	University of Toronto, Toronto
Akira Yamasaki	University of Manitoba, Winnipeg
Carla Jean Thompson	Fort Richmond Collegiate, Winnipeg

Waiting list/auditors

Sirirak Chantakru	Kasetsart University, Thailand
Dina Johar	Manitoba Institute of Cell Biology, Winnipeg
Karol McNeill	University of Manitoba, Winnipeg

Man Ying	Shaftsbury High School, Winnipeg

2005

Dr. Betty Engracia	De La Salle University, The Philipines.
Andrea Caporali	University of Parma, Italy.
Rahul Sarkar	University of Waterloo, Ontario.
Shauna Loewen	University of Manitoba, Winnipeg.
Heidi Dietrich	Delft University, The Netherlands.
Vasantha Atmuri	University of Manitoba, Winnipeg.
Dr. Sabine Hombach	University of Manitoba, Winnipeg.
Sherif Louis	University of Manitoba, Winnipeg.
Iris Gehrke	University of Manitoba, Winnipeg.
Jessica Gietz	University of Manitoba, Winnipeg.
Dr. Helen Tempest	University of Kent, UK.
Ana Carolina Laus	University of Sao Paulo, Brazil.
Antonietta Versace	Manitoba Institute of Cell Biology, Winnipeg.
Landon Wark	University of Manitoba, Winnipeg
Zhenyu Li	University of Manitoba, Winnipeg.
Dr. Fabien Kuttler	Manitoba Institute of Cell Biology, Winnipeg.
Richard Wiens	University of Manitoba, Winnipeg.
Dr. Reena Ray	Children's Hospital of Eastern Ontario, Ontario.
Juliana Cuzzi	University of Sao Paulo, Brazil.
Dr. Sinee Disthabanchong	Mahidol University, Thailand.

2005 Nov-December. 1st international workshop on FISH and SKY in Chiang Mai, Thailand.

Dr. Chotipa Sakulsingharoj	Department of Biology, Faculty of Science Maejo University, Chiang Mai.
Kanokkan Bumroongkit	Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai.
Dr. Aprichart Oranratnachai	Department of Obstetrics and Gynaecology, Faculty of Medicine, Chiang Mai University, Chiang Mai.
Chaniporn Puaninta	Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai.
Dr. Dalina Tanyong (Itchayanan)	Department of Clinical Microscopy, Faculty of Medical Technology, Mahidol University, Bangkok.
Khajornsak Tragoolpua	Division of Clinical Microbiology Department of Medical Technology Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai.
Dr. Kearkiat Praditpornsilpa	Division of Nephrology Department of Internal Medicine, King Chulalongkorn Memorial Hospital, Bangkok.
Dr. Krai Meemon	Department of Anatomy, Faculty of Science, Mahidol University, Bangkok.
Dr. Krissanapong Manotham	Renal Unit, Department of Medicine, Lerdsin General Hospital, 190, Silom, Bang-Rak.

Dr. Patthama Pongpom	Department of Microbiology, Faculty of Medicine, Chiang Mai University. Chiang Mai.
Dr. Piyanuch Boonkumklao (Niamsup)	Department of Biology, Faculty of Science, Maejo University, Sansai, Chiang Mai.
Dr. Prasit Phowthongkum	Faculty of Medicine, Chulalongkorn University, Bangkok.
Dr. Rutch Khattiya	Food animal clinic, Faculty of Veterinary Medicine, Chiangmai University, Mae-hia, Muang, Chiang Mai.
Dr. Supaporn Suwiwat	Department of Pathology, Faculty of Medicine, Prince of Songkla University, Hat-Yai, Songkla.
Dr. Surintorn Boonanuntanasarn	School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima.
Dr. Sutisa Nudmamud-Thanoi	Department of Anatomy, and Center for Central Facility and Research Development, Faculty of Medical Science, Naresuan University, Phitsanulok.
Dr. Suvara K. Wattanapitayakul	Department of Pharmacology, Faculty of Medicine, Srinakharinwirot University, Bangkok.
Dr. Tewin Tencomnao	Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok.
Dr. Tuangporn Suthiphongchai	Department of Biochemistry, Faculty of Science, Mahidol University, Bangkok.
Dr. Weerah Wongkham	Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai.

2006 (spring)

Molly Pind	U of Manitoba
Dr. Gregory Weitsman	MICB, U of Manitoba
Emilia Wiechec	University of Aarhus, Denmark
Dr. Yanmin Victor Yang	NRC and U of Manitoba
Francisco Mendoza	MICB, U of Manitoba
Dr. Soumya Panigrahi	MICB, U of Manitoba
Johannes Kohlschutter	University of Freiburg, Germany
Sherif Louis	MICB, U of Manitoba
Dr. Ludger Klewes	MICB, U of Manitoba
Aaron Sen	MICB, U of Manitoba
Dr. Raouf Fetni	Ste-Justine Hospital, Mc Gill Univ. and U of Montreal, Montreal
Macoura Gadji	U of Sherbrooke, Quebec
Nina Thiessen	NRC-IBD
Dr. Oumar Samassekou	U of Sherbrooke, Quebec
Sawcene Hazourli	U of Montreal, Quebec
Dr. Suradej Hongeng	Mahidol University, Bangkok, Thailand
Dr. Adriana Szeghalmi	U of Manitoba
Dr. Alex Gutsol	U of Manitoba
Lisa Manning	Canadian Food Inspection Agency/National Centre for Foreign Animal Disease

Dr. Esteban Alberti	International Center of Neurological Restoration, Havana, Cuba
Dr. Anna Szeles	Karolinska Institute, Sweden

TEACHING AND TRAINING

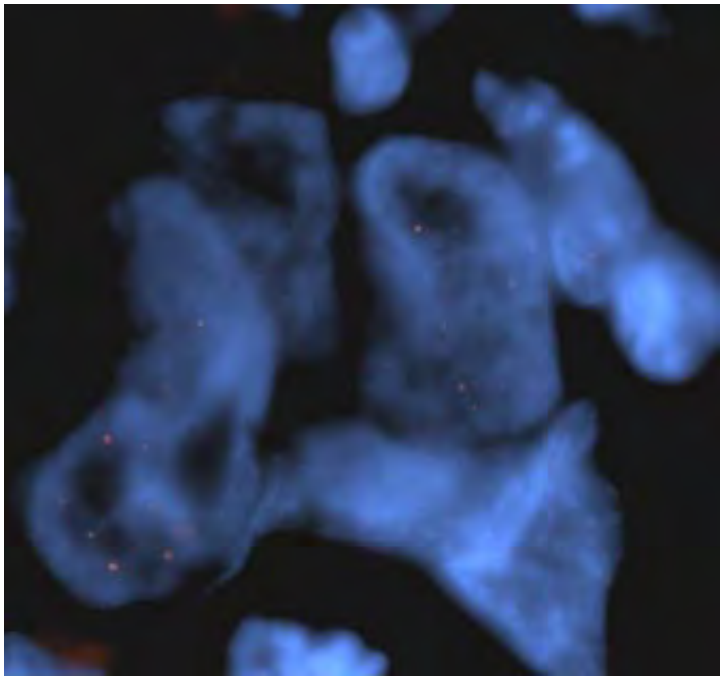
"Genomic Instability"	Cancer Biology (course #36.720)	1998-present
New Chromosome Technologies	Introduction to Human Genetics (course #125.302)	1998-2002
MICB and other Departments	Use of the FISH facility	1995-present
Fort Garry School Division No. 5	Science Curriculum for Genetics and Cancer Biology	
Teaching and Training of teachers and student (grades 10-12)		
Students of other schools	summer projects for highly motivated students who wish to start careers in science	
4th Year Radiation Oncologists	Seminars on 'RNA', 'DNA', 'Chromosomes' (December 1999)	
Resident Training	Research Project on 'Head and Neck' cancer (in collaboration with Drs. C. Birek and P. Kerr).	
Chiang Mai University (Thailand)	Co-supervision of 5 Ph.D. students. Laboratory courses and introduction to novel technologies. Visiting lecturer: 4/2000-5/2000.	
Nucleic Acids course (36.724)	2000-present	
CIHR Strategic Training Program	"Innovative Technologies in Multidisciplinary Health Research Training". Program funded for 6 years, started in 2002. Our training website: http://www.itmhrt.ca	

Organization of workshops and training

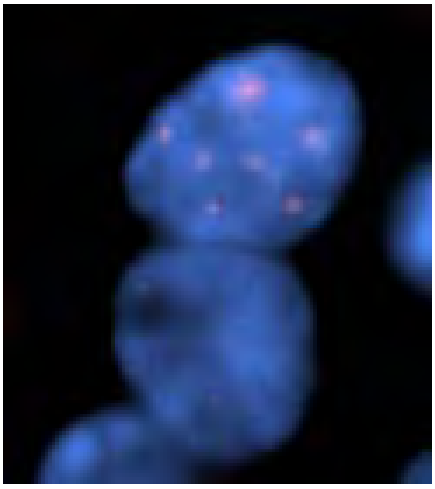
1) PCR and Cloning	November 1999
2) Spectral karyotyping	November 2000
3) Advanced Microscopy and Imaging	January 2001
4) Gateway Cloning	April 2001
5) Principles of FISH	September 2001
6) New technologies	October 2001 (Medical Hall of Fame students)
7) Development of CIHR Strategic Training Program entitled "Innovative Technologies in Multidisciplinary Health Research"	April 2001-present

8) CIHR Strategic training Program workshops since 2002. Details are listed on the training website (http://www.itmhrt.ca)

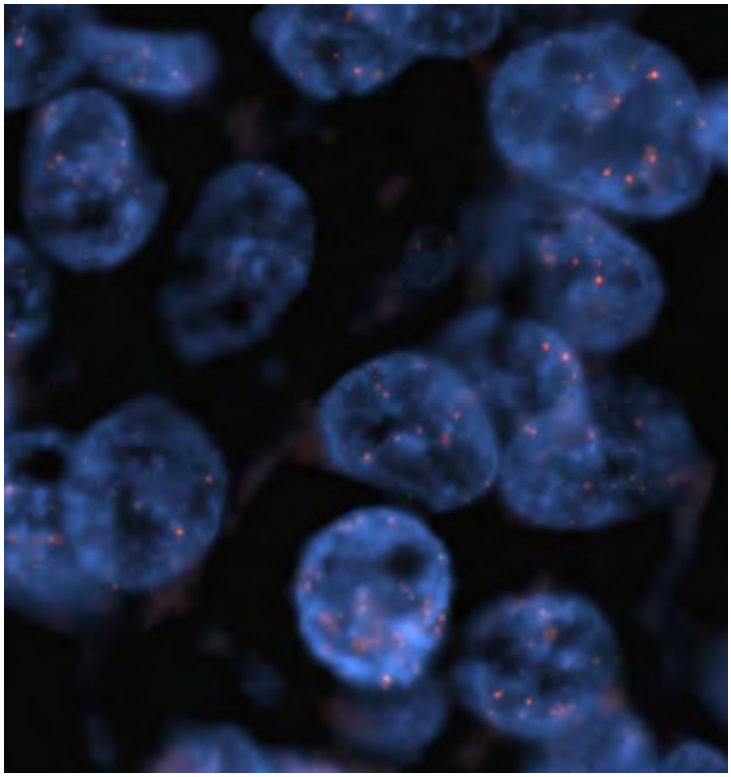
SUPPORTING DATA:



BRCA1-mutant carrier with breast cancer.



BRCA2-mutant carrier with breast cancer



non-BRCA1/2 carrier with breast cancer